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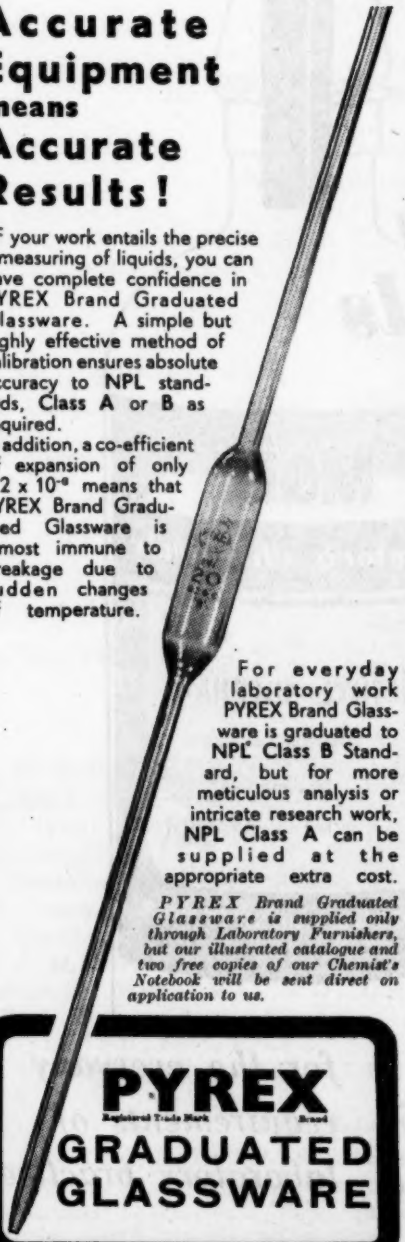

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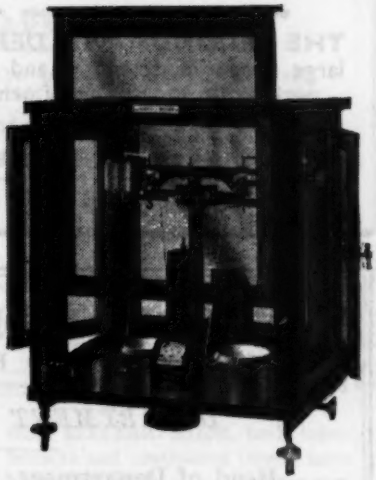

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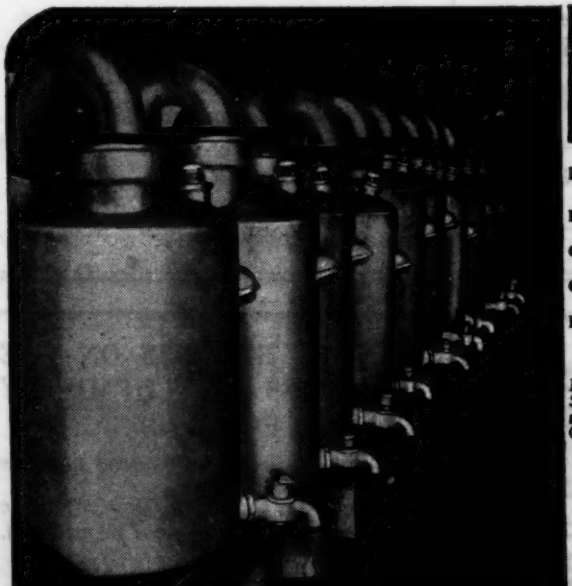

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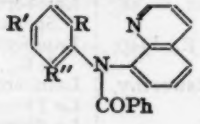
	18*	*for* "$C_{10}H_{18}O_4$" *read* "$C_{12}H_{22}O_4$."
1378	Table	Last figure in the sixth column. *For* 0.147 *read* 1.47.
1380	7	*for* "Peiss" *read* "Preiss".
1603	5*	Formulæ (III) should be



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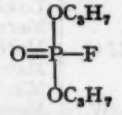
927		The formula to the left of (III.) should be



* From bottom.

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9. New compounds should be indicated by underlining the name at its first mention (excluding headings) both in the introduction and in the experimental section, and by giving analytical results in the form: " Found: ———. ——— requires ———." Analytical results for compounds which have previously been adequately described in the literature should be given in the form: " Found: ———. Calc. for ———: ———."

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Nominal Details.	Cost or Value when Acquired.	Market Value 31st December 1948.
	£ s. d.	£ s. d.
£3426 15s. 0d. 2½% Conversion Loan, 1961, or after	1884 12 8	2584 9 9
£3408 2s. 6d. 3% Funding Loan, 1959/69	2429 11 5	2504 9 0
£3400 14s. 9d. 3% Savings Bonds, 1960/70	2418 11 0	2484 15 3
£3000 3% Savings Bonds, 1965/75	2006 4 5	2060 0 0
£4490 19s. 8d. 3% British Transport Guaranteed Stock, 1978/88 ...	5444 14 11	4513 8 9
£3546 1s. 4d. 3% British Electricity Guaranteed Stock, 1968/73 ...	2549 4 5	2622 9 0
£1200 2½% Defence Bonds	1200 0 0	1200 0 0
£3400 2½% Bristol Corporation Debenture Stock, 1957	2070 2 0	2328 0 0
£1550 2½% London County Consolidated Stock, 1954/59	1639 2 6	1604 5 0
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£3500 3% Commonwealth of Australia Registered Stock, 1964/66 ...	2509 7 6	2575 0 0
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	£28,736 17 5	£29,042 15 7

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£9260 19s. 9d. 2½% Conversion Loan, 1961, or after	1566 16 4	2407 19 0
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£2261 1s. 11d. 3% Savings Bonds, 1960/70	2276 1 11	2340 4 8
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£2000 3% British Electricity Guaranteed Stock, 1968/73	2002 10 0	2060 0 0
£1200 3% Commonwealth of Australia Registered Stock, 1964/66 ...	1204 10 0	1236 0 0
	£9978 19 7	£11,275 14 7

SPECIAL RESERVE FUND.

£1300 2½% Defence Bonds	1300 0 0	1300 0 0
£2400 2½% National War Bonds, 1949/51	2400 0 0	2418 0 0
£3700 2½% National War Bonds, 1952/54	3700 0 0	3820 5 0
£1500 3% Savings Bonds, 1955/65	1500 0 0	1567 10 0
£2582 10s. 9d. 3% Liverpool Corporation Stock, 1955/58	2647 2 0	2672 18 6
£3000 2½% Funding Loan, 1956/61	3011 5 0	3060 0 0
	£14,558 7 0	£14,838 13 6

RESEARCH FUND.

£4177 16s. 1d. 2½% Conversion Loan, 1961, or after	3625 16 8	4449 7 3
£500 Defence Bonds, 4th issue	500 0 0	500 0 0
£4000 3% Savings Bonds, 1960/70	4060 0 0	4140 0 0
£3755 2s. 1d. 3% British Transport Guaranteed Stock, 1978/88 ...	3567 19 5	3773 17 7
£3719 8s. 2d. 3% British Electricity Guaranteed Stock, 1968/73 ...	3734 1 2	3830 19 9
£4000 3% Commonwealth of Australia Registered Stock, 1964/66 ...	4015 0 0	4120 0 0
	£19,492 17 3	£20,814 4 7

SPECIAL PUBLICATIONS FUND.

Nominal Details.	Cost or Value when Acquired	Market Value 31st December, 1948.
	£ s. d.	£ s. d.
£715 8s. 10d. 2½% Conversion Loan, 1961, or after	541 2 4	761 18 11
£746 1s. 0d. 3% British Transport Guaranteed Stock, 1978/88 ...	719 18 9	749 15 7
£750 3% British Electricity Guaranteed Stock, 1968/73	750 18 9	772 10 0
	£2011 19 10	£2284 4 6

STAFF PENSIONS FUND.

£683 6s. 1d. 2½% Conversion Loan, 1961, or after	527 13 9	727 14 4
£150 2½% National War Bonds, 1951/53	150 0 0	153 15 0
£170 3% Savings Bonds, 1955/65	170 0 0	170 0 0
£815 2s. 7d. 2½% Funding Loan, 1956/61	818 3 9	831 8 7
£2500 2½% Defence Bonds	2500 0 0	2500 0 0
	£4165 17 6	£4382 17 11

THE EDWARD FRANK HARRISON MEMORIAL TRUST FUND.

£61 15s. 8d. 2½% Conversion Loan, 1961, or after	50 1 5	65 15 11
£1489 10s. 6d. 3% British Transport Guaranteed Stock, 1978/88 ...	1051 6 6	1496 19 5
	£1101 7 11	£1562 15 4

SPECIAL LECTURES FUND.

£1414 15s. 3d. 3% British Transport Guaranteed Stock, 1978/88 ...	£1365 5 0	£1421 16 8

THE FARADAY LECTURE FUND.

£374 19s. 0d. 2½% Conversion Loan, 1961, or after	£369 4 0	£399 6 5

THE TILDEN LECTURE FUND.

£419 4s. 10d. 3% Funding Loan, 1959/69	386 12 10	436 0 3
186 National Provincial Bank Shares £5 each, £1 paid "B"	678 18 0	744 0 0
£681 19s. 1d. 3% British Transport Guaranteed Stock, 1978/88 ...	625 2 0	685 7 3
£220 3% Metropolitan Water Board (Kent Water Works) Deb. Stock	188 2 0	220 0 0
	£1878 14 10	£2085 7 6

CENTENARY FUND.

£2477 13s. 11d. 3% Savings Bonds, 1955/65	2491 11 10	2589 3 10
£2500 3% Savings Bonds, 1960/70	2534 7 6	2587 10 0
£2500 3% British Electricity Guaranteed Stock, 1968/73	2498 8 9	2575 0 0
£2279 13s. 0d. 3% British Transport Guaranteed Stock, 1978/88 ...	2237 7 9	2291 0 11
£A3500 4% Commonwealth of Australia Treasury Bonds, 1961 ...	2936 7 6	2912 0 0
	£12,698 3 4	£12,954 14 9

W. A. AKERS, *Treasurer.*

We have examined the above Balance Sheets and accompanying Income and Expenditure Accounts with the Books and Vouchers of the Society, and certify them to be in accordance therewith. We have also verified the Balances at the Bankers and Investments.

FINSBURY CIRCUS HOUSE,
BLOMFIELD STREET, E.C.2.
MARCH 21ST, 1949.

W. B. KERR & CO.
Chartered Accountants.

286. *The Chemistry of Simple Heterocyclic Systems. Part III Basic Centres of 4-Substituted Quinazoline Derivatives.*

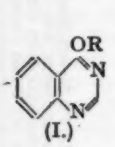
By J. S. Morley and J. C. E. Simpson.

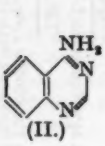
The preparation from 4-hydroxyquinazoline of 4-phenoxy- and 4-amino-quinazoline is described.

Fusion of 4-phenoxyquinazoline with methyl toluene-*p*-sulphonate yields 1-methyl-4-quinazolone *via* an unstable quaternary salt, thus disclosing $N_{(1)}$ as the basic centre in the phenoxy-compound; however, similar treatment of 4-methoxyquinazoline attacks both $N_{(1)}$ and $N_{(3)}$ with formation of the quaternary salt (IV) or (IV*a*).

Attempted conversion of cinnoline methiodide into 1-methyl-4-cinnolone results in dealkylation and formation of cinnoline in 50% yield.

It has recently been shown (Morley and Simpson, *J.*, 1948, 360) that 6- and 7-nitro-4-acetamido- and -4-phenoxy-quinazoline are converted by quaternary-salt formation and subsequent fission into 6- and 7-nitro-1-methyl-4-quinazolone, and therefore that $N_{(1)}$ is the proton-accepting centre in these quinazolines. On the other hand, the centre of basicity, as deduced from quaternary-salt formation and degradation, is $N_{(3)}$ in quinazoline itself (Gabriel and Colman, *Ber.*, 1904, **37**, 3643). It was therefore desirable to ascertain whether $N_{(1)}$ or $N_{(3)}$ is the basic centre in other 4-substituted quinazolines, and we now record our results with 4-*phenoxyquinazoline* (I; R = Ph) and 4-methoxyquinazoline (I; R = Me).


(I.)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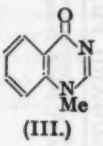
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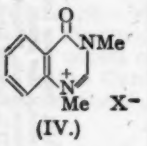
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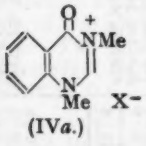
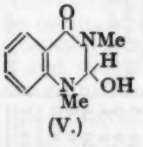

(IV*a*.)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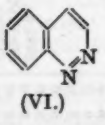
(VI.)

4-Phenoxyquinazoline was readily prepared from 4-chloroquinazoline by reaction with phenol and potassium hydroxide. Treatment of the chloro-compound with phenol and ammonium carbonate likewise yielded (I; R = Ph) together with some 4-*aminoquinazoline* (II), which was also obtained from 4-chloroquinazoline and concentrated aqueous ammonia at room temperature (cf. Tomisek and Christensen, *J. Amer. Chem. Soc.*, 1945, **67**, 2112) and, almost quantitatively, from the phenoxy-compound and ammonium acetate at 190°. The properties of compounds previously designated as 4-aminoquinazoline are not in good agreement with those of our material; thus Dewar (*J.*, 1944, 619) states that 4-aminoquinazoline, m. p. 259—260°, is almost non-basic and fails to react with acid chlorides under a variety of conditions, and Tomisek and Christensen (*loc. cit.*) state that it melts at 245—260° (decomp.) and yields (apparently with considerable difficulty) an acetyl derivative, m. p. 172°. In our hands, 4-aminoquinazoline prepared by each of the three methods mentioned has m. p. 267° (decomp.), readily yields 4-acetamidoquinazoline, m. p. 174—175°, and has pronounced basic properties (see Part IV, following paper).

Fusion of 4-phenoxyquinazoline with methyl toluene-*p*-sulphonate proceeded with elimination of phenol, and subsequent treatment with water yielded 1-methyl-4-quinazolone (III), isolated as the *picrate*. The structure of this base was proved by alkaline fission to *o*-methylaminobenzamide (*N*-methylanthranilamide) and also by nitration to 6-nitro-1-methyl-4-quinazolone (Morley and Simpson, *loc. cit.*). As 4-hydroxyquinazoline yields 3-methyl-4-quinazolone under ordinary conditions of alkylation (Knape, *J. pr. chem.*, 1891, **43**, 209; Bogert and Geiger, *J. Amer. Chem. Soc.*, 1912, **34**, 524), these results indicate that 4-phenoxyquinazoline is converted by reaction at $N_{(1)}$ into an unstable quaternary salt, which then undergoes decomposition to 1-methyl-4-quinazolone.

Bogert and Geiger (*J. Amer. Chem. Soc.*, 1912, **34**, 683) found that 4-methoxyquinazoline, 4-hydroxyquinazoline, and 3-methyl-4-quinazolone reacted with methyl iodide to give the same iodide, $C_{10}H_{11}ON_2I$, which must therefore be represented by one or other of the structures (IV) or (IV*a*) (X = I); its formation is thus of no value for the purpose of deciding whether $N_{(1)}$ or $N_{(3)}$

is the basic centre in 4-methoxyquinazoline. In the hope that the use of a different reagent might produce the desired result of quaternisation without further alkylation, 4-methoxyquinazoline was fused with methyl toluene-*p*-sulphonate (1 mol.), but, after conversion into the iodide, the sole product was, again, the dialkylated salt (IV or IV*a*; X = I). Treatment of this salt with cold alkali yielded an oil, the properties of which suggested that it might be the *pseudo*-base (V); however, the oil gave a *picrate*, m. p. 189°, which differed from the quaternary *picrate* (IV or IV*a*; X = $C_6H_2O_7N_3$), m. p. 198°, prepared from the iodide.

No decision, therefore, can be made as to the position of the basic centre in 4-methoxyquinazoline. The results described in this and an earlier paper (*J.*, 1948, 360), however, establish that the introduction of a phenoxy- or an acetamido-group into the quinazoline nucleus at C_4 produces a shift of the basic centre from $N_{(3)}$ to $N_{(1)}$. Particular interest thus attaches to the determination of the centre of basicity of the isomeric cinnoline (VI), inasmuch as $N_{(1)}$ has been shown to function in this capacity in 4-methyl- and 4-amino-cinnolines (Atkinson and Simpson, *J.*, 1947, 808; Simpson, *J.*, 1947, 1653). Preliminary attempts in this direction have been unsuccessful; oxidation of cinnoline methiodide with alkaline ferricyanide gave 50% of cinnoline, and no 1-methyl-4-cinnolone (Schofield and Simpson, *J.*, 1945, 512) could be detected; and treatment of the methiodide with alkali in an atmosphere of nitrogen gave no definite product.

Experimental.

Melting points are uncorrected.

4-Phenoxyquinazoline.—(*a*) 4-Chloroquinazoline (3·3 g.) was added during 10 minutes to a solution of potassium hydroxide (1·3 g.) in warm phenol (40 g.; temperature > 50°). After 1 hour at 50° the melt was digested with cold 2N-sodium hydroxide and the suspension extracted with ether. The product (4·01 g.; 90%) from the washed and dried extract was crystallised from ligroin (b. p. 60—80°), yielding *4-phenoxyquinazoline* as colourless plates, m. p. 78—79° (Found: C, 75·6; H, 4·6. $C_{14}H_{10}ON_2$ requires C, 75·65; H, 4·5%).

(*b*) 4-Chloroquinazoline (1 g.) was added with powdered ammonium carbonate (4 g.) to phenol (10 g.) at 90°. After ½ hour the product was worked up as in (*a*). It was not wholly soluble in ether, and the insoluble fraction, after dissolution in 0·5N-hydrochloric acid and reprecipitation with ammonia, yielded 4-aminoquinazoline (0·17 g.), m. p. 265—267° (decomp.) alone and mixed with the material described below. The ether-soluble portion of the reaction-product gave 4-phenoxyquinazoline (0·7 g.).

4-Aminoquinazoline.—(*a*) 4-Phenoxyquinazoline (1 g.) was added to freshly-fused ammonium acetate (5 g.) at 160°; the temperature of the mixture was then raised to 190° and kept there for 2 minutes. When cold, the mass was dissolved in water (10 c.c.) and made alkaline with 2N-sodium hydroxide. Pure *4-aminoquinazoline* (0·64 g.; 98%) separated in colourless needles, m. p. 267—268° (decomp.), unchanged by crystallisation from alcohol; it could not be obtained anhydrous even after prolonged drying at 80° in a vacuum (Found: C, 63·8; H, 4·85; N, 28·4. $C_8H_7N_3,\frac{1}{2}H_2O$ requires C, 63·6; H, 5·1; N, 27·8%).

(*b*) 4-Chloroquinazoline (1 g.) and aqueous ammonia (50 c.c.; *d* 0·88) after 10 days at room temperature gave a crude product (0·8 g., m. p. 202—215°) which, after dissolution in warm 0·5N-hydrochloric acid, filtration, and reprecipitation, yielded fairly pure 4-aminoquinazoline (0·59 g.; 67%), m. p. 245—251° (decomp.). This (0·5 g.) was suspended in water (80 c.c.), treated with sufficient acetic acid to effect dissolution, and basified with ammonia; the solution, after filtration, in the hot, from a little gelatinous material, deposited glittering needles of pure 4-aminoquinazoline, m. p. 265—267° (decomp.).

4-Acetamidoquinazoline (60 mg.) was prepared by heating the base (100 mg.) with acetic anhydride (1 c.c.) under reflux for 2 hours, and then adding dry ether (5 c.c.) to the cooled solution; it formed fine, colourless needles, m. p. 174—175° (Found: C, 64·0; H, 4·7; N, 22·5. Calc. for $C_{10}H_9ON_3$: C, 64·15; H, 4·85; N, 22·45%).

*Reaction of 4-Phenoxyquinazoline with Methyl Toluene-*p*-sulphonate.*—A mixture of 4-phenoxyquinazoline (3·52 g.) and methyl toluene-*p*-sulphonate (3·2 g.) was fused at 150° (bath temperature) for ½ hour; a strong odour of phenol developed and a clear orange-red melt was formed. When the mixture had cooled, water (25 c.c.) was added, and the mixture extracted with ether; the ether-soluble material was identified by conversion into tribromophenol (4·4 g.; 84%), m. p. 94—95°. Treatment of the aqueous solution with picric acid (4 g.) in hot ethanol (80 c.c.) gave *1-methyl-4-quinazolone picrate* (5·37 g.; 87%), which formed yellow needles, m. p. 249—250° (decomp.), from methanol, in which it was sparingly soluble (Found: C, 46·5; H, 3·05; N, 18·0. $C_{15}H_{11}O_8N_5$ requires C, 46·3; H, 2·85; N, 18·0%).

The free base (crude, 1·25 g.), obtained from the picrate (5·3 g.) by decomposition with ice-cold ammonia in the presence of chloroform, formed a hydrate, m. p. 65—66°, converted into the hygroscopic anhydrous base (long colourless needles, m. p. 141—142°, from chloroform–ligroin) by drying at 80—90° for 3 hours. The ammoniacal solution, after the removal of chloroform, was treated with 20% aqueous sodium hydroxide and kept for 24 hours at room temperature; extraction with benzene then yielded *o*-methylaminobenzamide (0·36 g.) (see below). Decomposition of the picrate with lithium hydroxide or sodium hydroxide gave poorer yields of the quinazolone. When a solution of the quinazolone (0·15 g.) in cold water (2 c.c.) was boiled for 2 minutes with 2N-sodium hydroxide (2 c.c.), some ammonia was evolved, and *o*-methylaminobenzamide (40 mg.) separated on cooling, m. p. 162—163° (Knape, *J. pr. Chem.*, 1891, **43**, 209, gives m. p. 159—160°), after crystallisation from aqueous alcohol (Found: C, 63·8;

H, 6·5. Calc. for $C_8H_{10}ON_2$: C, 64·0; H, 6·7%). The quinazolone (100 mg.) was added in portions to a mixture of nitric acid (0·5 c.c.; *d* 1·50) and concentrated sulphuric acid (0·5 c.c.) at 25—30°. After 20 minutes the solution was poured on ice, made just alkaline with solid potassium carbonate, and filtered, and the filtrate was evaporated under reduced pressure, yielding 6-nitro-1-methyl-4-quinazolone (30 mg.), m. p. 270—272° alone and mixed with an authentic specimen (*J.*, 1948, 360).

3-Methyl-4-quinazolone.—The method of Bogert and Geiger (*J. Amer. Chem. Soc.*, 1912, **34**, 524) gave the base in 85% yield, but the yield was somewhat lower when methyl sulphate and excess of alkali were used. The anhydrous base, m. p. 105—106° (Bogert and Geiger give m. p. 105°), readily reverted to the hydrate, m. p. 71—72° (Bogert and Geiger give m. p. 70—71°); both forms gave large depressions in m. p. when mixed with the corresponding specimens of 1-methyl-4-quinazolone. The *picrate*, m. p. 215—216° (depressed by 1-methyl-4-quinazolone picrate), formed soft, yellow needles from alcohol, in which it was much more soluble than the 1-methyl isomer (Found: C, 46·6; H, 3·05; N, 18·1. $C_{15}H_{11}O_8N_5$ requires C, 46·3; H, 2·85; N, 18·0%).

Reaction of 4-Methoxyquinazoline with Methyl Toluene-p-sulphonate.—4-Methoxyquinazoline (0·64 g.) and methyl toluene-*p*-sulphonate (0·8 g.) were heated together for a few minutes at 160°. The colourless crystalline mass obtained on cooling was dissolved in warm water (2 c.c.) and treated with a solution of potassium iodide (1 g.) in water (2 c.c.), yielding the iodide (1·01 g.; 83%) as colourless needles, m. p. 275—277° after sintering at 265° [Bogert and Geiger, *loc. cit.*, give m. p. 275° (corr.)] (Found: C, 39·75; H, 3·7; N, 9·7. Calc. for $C_{10}H_{11}ON_2I$: C, 39·75; H, 3·7; N, 9·3%). The corresponding *picrate*, obtained from the iodide and picric acid in aqueous solution, crystallised from water in soft yellow plates, m. p. 197—198° (Found: C, 47·65; H, 3·35; N, 17·35. $C_{16}H_{13}O_8N_5$ requires C, 47·65; H, 3·25; N, 17·4%). A solution of the iodide (0·2 g.) in water (5 c.c.) was treated with 2N-sodium hydroxide (5 c.c.) and left overnight. A colourless oil (insoluble in water, readily soluble in dilute acids and in ether, benzene, or chloroform) had then separated; this (0·13 g.) was isolated by extraction with ether and converted into a *picrate* (0·25 g.), which separated from alcohol in yellow needles, m. p. 188—189° (153—155° when mixed with the picrate, m. p. 197—198°, described above) (Found: C, 46·4; H, 3·85; N, 18·2, 17·9, 18·3. $C_{16}H_{13}O_8N_5,\frac{1}{2}H_2O$ requires C, 46·6; H, 3·4; N, 17·0%).

Alkaline Decomposition of Cinnoline Methiodide.—(*a*) N-Sodium hydroxide (5 c.c.) and a solution of cinnoline methiodide (0·4 g.) in water (10 c.c.) were added alternately, in small portions, to a solution of potassium ferricyanide (1·2 g.) in water (10 c.c.) during 5 minutes; the deep emerald-green solution thus formed was kept at room temperature for ½ hour with occasional shaking. Addition of excess of 20% sodium hydroxide and extraction with chloroform gave cinnoline, isolated as its picrate (0·26 g.; 49%), m. p. and mixed m. p. 196—197° (decomp.), and further authenticated by reconversion into the methiodide [m. p. and mixed m. p. 171—173° (decomp.)] with warm methanolic methyl iodide.

(*b*) Cinnoline methiodide (0·2 g.) and 0·5N-sodium hydroxide (5 c.c.) were set aside under nitrogen for ½ hour at room temperature. The deep-blue suspension was filtered into a separating funnel under nitrogen, giving a residue (A); the filtrate was treated with excess of 20% sodium hydroxide and extracted with benzene, but only a trace of oil was obtained on evaporation. The blue-green residue (A) (0·1 g., m. p. 120—125° (decomp.)) was almost insoluble in water and in benzene, but dissolved in dilute mineral acids giving pale red solutions from which it was reprecipitated by ammonia or caustic alkalis; it showed no tendency to form a picrate.

We are indebted to the Medical Research Council for a Research Studentship (J. S. M.).

WARRINGTON YORKE DEPARTMENT OF CHEMOTHERAPY,
LIVERPOOL SCHOOL OF TROPICAL MEDICINE. [*Received, August 27th*, 1948.]

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287. *The Chemistry of Simple Heterocyclic Systems. Part IV. Basic Strengths of Some 4-Substituted Cinnolines, Quinazolines, and Quinolines.*

By J. R. KENEFORD, J. S. MORLEY, J. C. E. SIMPSON, and P. H. WRIGHT.

The basic strengths have been determined potentiometrically for a series of quinoline, quinazoline, and cinnoline derivatives containing various 4-substituents. For the parent bases, and for compounds containing the groups OH, Cl, OPh, and OMe, the order of basicity is quinoline > quinazoline > cinnoline, which is the reverse of the observed order of strengths, as acids, of the hydroxy-compounds. For amino- and anilino-compounds, however, the order is quinoline > cinnoline > quinazoline, and chemical evidence indicates that the transposition is the result of abnormally low values for the quinazoline derivatives.

For each heterocyclic type, basicity diminishes in the order 4-NH_2 > NHPh > 6-NO_2-4-NH_2 > 4-OMe > OPh > Cl > OH. The effect of methoxyl is base-strengthening in the quinoline and cinnoline series (compared with the unsubstituted compounds), but is base-weakening in the quinazoline nucleus.

THE determination of the pK_a values of some 4-amino-cinnolines, -quinazolines, and -quinolines (Morley and Simpson, this vol., p. 1014) disclosed the interesting fact that the order of basicity of the 6-nitro-4-amino-derivatives, namely, quinoline > cinnoline > quinazoline, is not the reverse of the presumed order of strengths, as acids, of 4-hydroxy-compounds, *viz.*, cinnoline > quinazoline > quinoline, as judged by qualitative observations. The pK_a determinations

recorded below were made partly in order to investigate this apparent anomaly, and partly in order to compare certain reactions (unpublished) of 4-substituted cinnolines, quinazolines, and quinolines with their strengths as bases or acids.

The values given in Tables I and II were all obtained in 50% aqueous alcohol by the potentiometric method, using the conditions already described (*loc. cit.*). The equation previously referred to (*loc. cit.*) was used in order to make the method applicable to weak bases, and three independent determinations were made on the weakest base (4-hydroxycinnoline, Table I) in order to form some idea of the degree of uncertainty involved in such cases. Of numerous determinations by different workers, the only values for a single compound which differed significantly from each other were those for 4-methoxyquinazoline, and we are unable to account for the exceptional behaviour of this compound. Table I gives values for a complete set of bases of the triple heterocyclic series, and Table II shows values for some miscellaneous quinoline and cinnoline compounds. The values given in Table III were obtained by titrating the hydroxy-compounds in 50% aqueous alcohol with sodium hydroxide, applying (to the value for 4-hydroxyquinoline) the corresponding correction for a weak acid.

TABLE I.

Basic strengths of 4-substituted quinolines, quinazolines, and cinnolines in 50% aqueous alcohol.

pK_a values and concentrations. [c = (mols./litre) × 10^3].

	Quinoline.		Quinazoline.		Cinnoline.	
Substituent.	pK_a.	c.	pK_a.	c.	pK_a.	c.
					·77	4·93
4-OH	2·41	4·96	2·07	4·93	1·66 (*a*)	4·93
					1·72 (*a*)	4·93
4-Cl	2·59	5·26	2·10 (*e*)	5·62	2·08	5·47
4-OPh	4·42 (*b*)	5·16	2·44 (*e*)	4·08	2·27	4·05
						14·59
H	5·0 (*c*)	—	3·26 (*d*)	6·96	2·51 (*f*)	6·18
			2·49 (*b*)	3·62		4·96
4-OMe	5·35 (*b*)	5·16	2·73	6·61	2·71	3·62
			2·91	7·89		
6-NO_2-4-NH_2	6·41 (*e*)	4·45	3·71 (*e*)	2·94	5·08 (*e*)	3·60
4-NHPh	7·52	5·00	4·65	5·00	5·31	5·00
4-NH_2	8·47	6·58	5·17 (*e*)	6·44	6·26 (*e*)	4·72

Determinations were made at 21—22° unless otherwise stated.
(*a*) At 15°. (*b*) At 18—19°. (*c*) Pouterman and Girardet, *Experientia*, 1947, **3**, 28. (*d*) Elderfield, Williamson, Gensler, and Kremer, *J. Org. Chem.*, 1947, **12**, 405, give 3·2 in 50% aqueous alcohol at 27°. (*e*) At 25°. (*f*) Titration of free base gave 2·50; titration of hydrochloride with sodium hydroxide gave 2·52.

TABLE II.

Basic strengths of 4-substituted quinolines and cinnolines in 50% aqueous alcohol.

pK_a values and concentrations at 25° ± 2°.

	Quinoline.		Cinnoline.	
Substituent.	pK_a.	c.	pK_a.	c.
4-Cl-6-NO_2	2·13	3·66	—	—
4-Cl-8-NO_2	2·64	3·90	—	—
8-NO_2-4-OPh	2·94	2·10	2·49	2·07
6-NO_2-4-OPh	3·25	2·09	3·15	2·14
8-NO_2-4-NH_2	6·63	3·92	—	—
4 : 6-$(NH_2)_2$	—	—	6·86 (*a*)	4·10

(*a*) Titration of hydrochloride with sodium hydroxide.

TABLE III.

Acid strengths of 4-hydroxy-compounds in 50% aqueous alcohol.

pK_a values and concentrations at 21°.

Quinoline.		Quinazoline.		Cinnoline.	
pK_a.	c.	pK_a.	c.	pK_a.	c.
12·43	6·21	9·98	6·16	9·53	6·16

Examination of these results leads to the following conclusions.

(i) For a given arrangement of substituents, the quinoline is invariably (in both Tables I and II) a stronger base than the cinnoline derivative.

(ii) Broadly speaking, the difference between the pK_a value of a quinoline and that of the analogous quinazoline or cinnoline increases as the pK_a values themselves increase. This effect, however, is only qualitative; that is to say, a smooth curve is not obtained if, for example, values for $(pK_{a(\text{quinoline})}-pK_{a(\text{cinnoline})})$ are plotted against $pK_{a(\text{quinoline})}$.

(iii) For 4-hydroxy-, 4-chloro-, 4-phenoxy-, and 4-methoxy-compounds, as well as for the unsubstituted bases, the relative order of basicity is quinoline > quinazoline > cinnoline [*i.e.*, the reverse of that shown by the hydroxy-compounds as acids (Table III)], but for the amino- and anilino-compounds the order is quinoline > cinnoline > quinazoline (Table I). It thus follows that the replacement of one of the "normal" groups (OH, Cl, OPh, OMe) by an "abnormal" group (NH_2, NHPh) has imposed a significant fine-structural change on either the cinnoline or the quinazoline derivatives. We have observed (unpublished results) that, among 4-amino- and 4-substituted-amino-derivatives of each type, the quinazoline compounds alone are hydrolysed in acid solution to the hydroxy-compounds; the fine-structural change in question may, therefore, be associated with the aminoquinazolines.

(iv) The order of effectiveness in promoting basicity of the various 4-substituents, *viz.*, 4-NH_2 > NHPh > 6-NO_2-4-NH_2 > 4-OMe > OPh > Cl > OH, is the same in each of the three heterocyclic series, but the effect of a methoxyl group, relative to that of a hydrogen atom, is different in the three series, 4-methoxy-quinoline and -cinnoline being stronger, and 4-methoxy-quinazoline weaker, bases than the unsubstituted compounds. The base-weakening effect of a 4-methoxyl group attached to a quinazoline nucleus is also apparent if the pK_a value of 8-amino-quinazoline (2·49) is compared with that of 8-amino-4-methoxyquinazoline (2·31) (Elderfield, Williamson, Gensler, and Kremer, *J. Org. Chem.*, 1947, **12**, 405). However, the differences involved are too small to justify discussion of their possible significance.

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288. *Deoxy-sugars. Part IV. A Synthesis of 2-Deoxy-D-ribose from D-Erythrose.*

By W. G. OVEREND, M. STACEY, and L. F. WIGGINS.

The synthesis of 2-deoxy-D-ribose from D-erythrose as the initial material is described. Several methods of obtaining D-erythrose are outlined, and a method for its purification is discussed.

THE difficulty in preparing 2-deoxy-D-ribose, the deoxypentose of deoxyribonucleic acid, and hence a sugar of great biological importance, has hitherto prevented a systematic study of its derivatives. We have now studied many possible methods of its synthesis. Two of the methods investigated have already been reported. One concerned a study of the catalytic reduction of 2-bromo β-methylarabinoside, which was obtained, together with 3-bromo β-methylxyloside, by treating 2 : 3-anhydro-β-methyl-D-riboside with hydrogen bromide, and the other involved the methanesulphonyl derivatives of D-arabopyranoside (Parts II and III).

Recently, Fischer and Sowden (*J. Amer. Chem. Soc.*, 1947, **69**, 1048) showed that when D-arabinose is treated with nitromethane and an acetylating agent it yields 1-nitro-D-*arabo*-3 : 4 : 5 : 6-tetra-acetoxyhex-1-ene, reduction of which gave 1-nitro 1 : 2-dideoxy-D-arabohexityl tetra-acetate (1-nitro-3 : 4 : 5 : 6-tetra-acetoxyhexane). Treatment of this compound with sodium hydroxide and then dilute sulphuric acid resulted in simultaneous elimination of the nitro-group and deacetylation and afforded 2-deoxy-D-glucose. It was apparent that if D-erythrose were used in the synthesis instead of D-arabinose, then a route to the synthesis of 2-deoxy-D-ribose would be provided. Consequently, various methods of making D-erythrose from easily accessible carbohydrates have been investigated.

Several methods have been described for the preparation of L-erythrose. Deulofeu and Selva (*J.*, 1929, 225) summarised methods which have been used to degrade L-arabinose to L-erythrose and outlined their own extensions of these methods, these being directed mainly towards

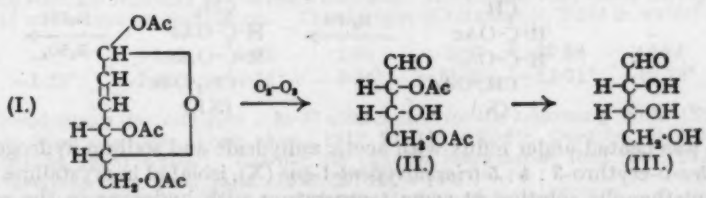
degrading L-arabonic acid by Ruff's method. More recently, Felton and Freudenberg (*J. Amer. Chem. Soc.*, 1935, **57**, 1637) treated L-arabinal with ozonised oxygen and obtained L-erythrose, and DL-erythrose has been prepared by the reduction of DL-erythronolactone by Glattfeld and Kribben (*ibid.*, 1939, **61**, 1720). There is very little information in the literature about methods of preparing the D-isomer.

When starch is oxidised by periodic acid it gives a polymeric dialdehyde (Jackson and Hudson, *ibid.*, 1937, **59**, 2049) which when hydrolysed with hydrochloric acid yields glyoxal and D-erythrose. [When methanolic hydrogen chloride is used for the hydrolysis, then one of the isomeric hexahydro-3 : 5-dimethoxy-2-(1-methyl-2- or 3-erythrofuranosyloxy)-furo-(3 : 4)-*p*-dioxins is also obtained (Michell and Purves, *ibid.*, 1942, **64**, 585).] Jackson and Hudson (*ibid.*, 1938, **60**, 989) did not isolate free D-erythrose, but oxidised the hydrolysis mixture with bromine-water, thereby obtaining oxalic acid (from the oxidation of glyoxal) and D-erythronic acid, isolated as its brucine salt. This was converted into D-erythronolactone obtained in an overall yield from starch of 7% of the theoretical. We have repeated this method in an attempt to obtain, in improved yield, a suitable derivative of D-erythrose from starch.

Potato starch was oxidised at 20—25° with an aqueous solution of periodic acid. The course of the oxidation was followed titrimetrically and was complete in 6 days. The oxidised starch was a coarse white powder, strongly reducing to Fehling's solution in the cold. It was hydrolysed with N-hydrochloric acid at 98—99° for 16 hours, and the hydrolysate then oxidised with bromine-water, thereby forming D-erythronic acid, isolated as the brucine salt in a yield of 33% of the theoretical: Hudson and Jackson (*loc. cit.*) obtained a 22% yield. It was found that D-erythronolactone could readily be purified through its 2 : 3-diacetyl derivative, although the overall yield of 2 : 3-diacetyl D-erythronolactone from starch was not much greater than that obtained by Hudson and Jackson (*loc. cit.*). Since this yield was so unsatisfactory attempts were made to utilise compounds derived from D-glucose.

Calcium D-gluconate was degraded to D-arabinose essentially by Hockett and Hudson's method (*ibid.*, 1934, **56**, 1632). Oxidation of this pentose with bromine-water gave D-arabonic acid (isolated as its calcium salt) in good yield. Hockett and Hudson's method of degradation (*loc. cit.*) was applied to calcium D-arabonate and it yielded D-erythrose as a pale yellow immobile syrup, which strongly reduced Fehling's solution. It could not be induced to crystallise or to form a solid phenylosazone. Purification was effected by converting it into 2 : 3-*iso*propylidene αβ-methyl-D-erythroside ($[\alpha]_D^{14\cdot5°}$ −55·5° in chloroform) by treatment with dry acetone and methyl alcohol containing 0·2% sulphuric acid and anhydrous copper sulphate. This erythroside was a pale yellow liquid which was purified by distillation in a vacuum as described by Felton and Freudenberg (*loc. cit.*) for purifying the L-isomer. Its hydrolysis with 0·1N-sulphuric acid at room temperature resulted in scission of the *iso*propylidene group and elimination of the glycoside residue, giving D-erythrose as a pale yellow syrup which was completely soluble in ethyl alcohol and which now formed a solid phenylosazone. D-Erythrose shows mutarotation in aqueous solution, equilibrium at −19·1° being reached after 73 hours (initial value −1·2°). At various times different values (see p. 1361) have been reported for the rotation of erythrose, and it seems that the true rotation of D-erythrose cannot be determined until the compound has been obtained in the crystalline state.

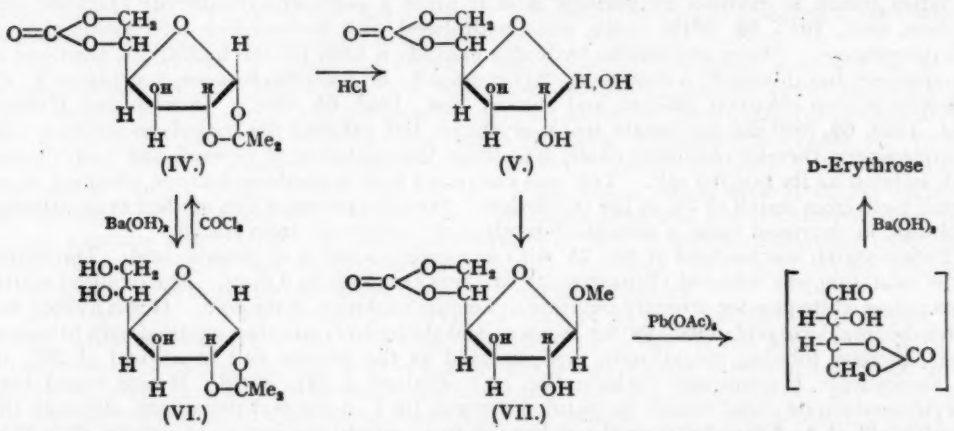
Two other methods of preparing D-erythrose from compounds derived from D-glucose were also carried out. It was shown by Bergmann, Kobel, Schotte, Rennert, and Ludewig (*Annalen*, 1923, **434**, 79) that when triacetyl glucal is boiled with water, the ethylenic linkage between C_1 and C_2 migrates to the position between C_2 and C_3 and acetylation of the product (later shown



to be diacetyl *pseudo*glucal; Bergmann, *ibid.*, 1925, **443**, 223) with acetic anhydride and sodium acetate gives triacetyl *pseudo*glucal (I). This was prepared (for physical constants, see p. 1362) and submitted to ozonisation. Scission of the ethylenic linkage occurred and there was obtained syrupy 2 : 4-*diacetyl* aldehydo-D-*erythrose* (II), hydrolysis of which with hydrochloric acid gave D-erythrose ($[\alpha]_D^{1\cdot5°}$ −19·7°, equilibrium) which was sufficiently pure readily to form a

solid phenylosazone. It was shown incidentally that catalytic hydrogenation of triacetyl *pseudo*glucal gives 1 : 4 : 6-*triacetyl* 2 : 3-*dideoxyglucopyranose*.

In another route to erythrose, treatment of glucose in suspension in dry acetone with carbonyl chloride gave 1 : 2-*iso*propylidene glucofuranose 5 : 6-carbonate (IV) (Haworth and Porter, *J.*, 1929, 2801). Since it can be prepared similarly from 1 : 2-*iso*propylidene

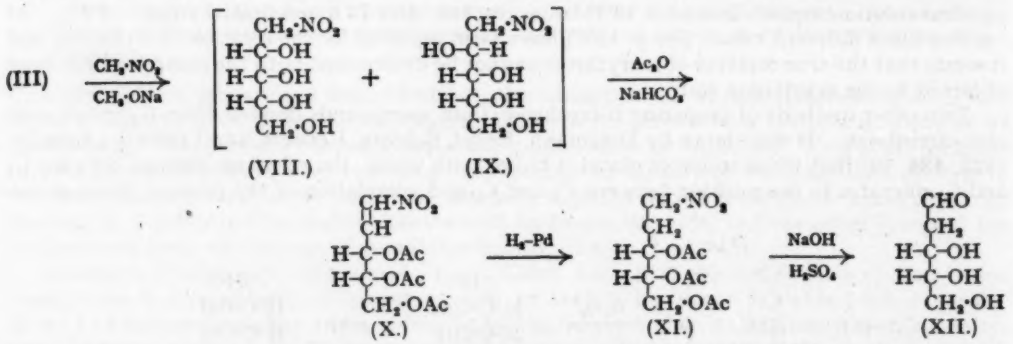


(IV.) (V.) (VI.) (VII.)

glucofuranose (VI), its structure follows. The action of hydrochloric acid on (IV) resulted in scission of the *iso*propylidene group and gave glucofuranose 5 : 6-carbonate (V). Treatment of this with methyl alcohol containing sulphuric acid afforded β-methylglucofuranoside 5 : 6-carbonate (VII) (cf. Haworth and Porter, *loc. cit.*). This was oxidised with excess of lead tetra-acetate, and the syrupy reaction product was heated with barium hydroxide solution, yielding D-erythrose ($[\alpha]_D^{18°}$ −17·6°, equilibrium), but only in low yield. Of the four methods outlined for the preparation of D-erythrose, the best yield was afforded by the degradation of calcium D-arabonate by Ruff's procedure.

It was of interest to find that (IV) formed crystalline 3-*methanesulphonyl* 1 : 2-iso*propylidene glucofuranose* 5 : 6-*carbonate* (m. p. 136—137°) when treated with methanesulphonyl chloride in dry pyridine, though as expected, treatment of this with dry acetone and sodium iodide in a sealed tube at 110° for 5 hours failed to give direct replacement of the methanesulphonyl group by an iodine atom.

D-Erythrose (III) was treated with nitromethane and sodium methoxide, giving a mixture of D-*ribo*- and D-*arabo*-1-nitropentitol (VIII and IX, respectively). These were not separated,



(VIII.) (IX.) (X.) (XI.) (XII.)

but the mixture was heated under reflux with acetic anhydride and sodium hydrogen carbonate and yielded 1-*nitro*-D-erythro-3 : 4 : 5-*triacetoxypent*-1-*ene* (X), isolated in crystalline state. This was reduced in methanolic solution at room temperature with hydrogen in the presence of a palladium-black catalyst, and then treated with sodium hydroxide followed by dilute sulphuric acid. The resulting syrup was heated with aniline in ethyl alcohol and water, and on standing 2-deoxy-D-ribose anilide was deposited. De-anilination was effected by treating the anilide with 0·5% oxalic acid solution in water, and there was obtained 2-deoxy-D-ribose (XII); anilination of a sample of this re-afforded the anilide.

Experimental.

Oxidation of Starch with Periodic Acid.—A solution of periodic acid in water (0·066 g. per c.c. of solution) was prepared by treating a solution of potassium metaperiodate (75·0 g.) in water (2·5 l.) with a solution of barium acetate monohydrate (89·12 g.) in water (500 ml.) at 70°. The mixture was kept at 5° for 6 hours, and was stirred continuously. Barium metaperiodate separated as a white powder, and was collected by filtration, washed with water until free from acetic acid, and then dried, first in air for 2 days and then at 110°. This product (50 g.) was suspended in water (90 c.c.) and 1·06N-sulphuric acid was added. Removal of the barium sulphate formed yielded an aqueous solution of periodic acid.

Freshly dried starch (13·5 g.) was added to the above periodic acid solution (282 c.c. = 18·8 g. HIO_4) and the mixture was shaken at 20—25°. The course of the oxidation was followed volumetrically and was complete in 144 hours. The polymeric dialdehyde was collected by filtration and washed with cold water to remove excess of periodic and iodic acids. The oxidation product was dried at 35° and was a white powder, coarse to the touch and strongly reducing in the cold to Fehling's solution; $[\alpha]_D^{24°}$ +9·5° (*c*, 0·209 in water): Hudson and Jackson (*J. Amer. Chem. Soc.*, 1937, **59**, 2049) give $[\alpha]_D^{20}$ +9°.

Hydrolysis of Oxidation Product.—The above product (12·5 g.) was heated with water (500 c.c.) at 100° for 3 hours, and the solution filtered. N-Hydrochloric acid (75 c.c.) was added to the filtrate which was diluted to 750 c.c. and kept at 98—99° for 16 hours, in a stoppered flask. The course of the hydrolysis was followed polarimetrically, but this procedure became increasingly difficult towards the end of the reaction as the solution became strongly coloured. After treatment with charcoal, bromine (20 ml.) was added, and the solution was kept for 4 days at room temperature in the dark. Excess of bromine was removed by aeration and the solution was neutralised with barium hydroxide. The barium oxalate formed was removed by filtration and excess of barium ions in the filtrate was removed by addition of 1N-sulphuric acid. Chloride ions present were removed with silver carbonate, and then silver ions in solution were precipitated by passage of hydrogen sulphide, excess of which was removed by boiling. When all extraneous ions had been removed from the solution, it was concentrated under diminished pressure to 200 c.c., and ethyl alcohol (50 c.c.) was added together with excess of brucine (50 g.). The solution was heated on a steam-bath for 3 hours and then the excess of brucine was extracted with chloroform. The aqueous residue was evaporated to a thick syrup, which on trituration with ethyl alcohol yielded amorphous brucine erythronate (15·2 g., 33%) which could not be induced to crystallise.

2 : 3-*Diacetyl* D-*Erythronolactone.*—Crude brucine D-erythronate (3·0 g.) was treated with excess of oxalic acid, the liberated brucine was extracted with chloroform, and excess of oxalate ions removed by adding calcium hydroxide. The filtered solution was evaporated to dryness under diminished pressure. The residue was added at 0° to freshly distilled acetic anhydride (25 c.c.) containing dry hydrogen chloride (0·6 g.), and the mixture shaken. The temperature was gradually allowed to increase to room temperature and kept thereat for 3 hours. Excess of acetic anhydride was removed by distillation under diminished pressure, and the residue was distilled as a colourless syrup, b. p. 200° (bath temp.)/12 mm., which slowly crystallised. After recrystallisation from hot water, it had m. p. 50—51·5°; yield 0·22 g. (22%). Glattfeld and Kribben (*loc. cit.*) give m. p. 53° for diacetyl DL-erythronolactone.

Calcium D-*Arabonate.*—D-Arabinose (117·40 g.) was dissolved in water (588 c.c.), and bromine (78·4 c.c.) added. The solution was kept for 2 hours, and then the excess of bromine was removed by aeration. The hydrogen bromide formed during the reaction was removed by adding silver oxide, and filtering off the precipitated silver bromide. Any silver in solution was precipitated with hydrogen sulphide, and the solution was boiled with calcium carbonate for 15 minutes. The filtered solution was evaporated to give a crystalline residue, which recrystallised from water in white needles (123·4 g.; 85·2%); m. p. 99—101°, $[\alpha]_D^{21°}$ −6·8° (*c*, 2·645 in water).

D-*Erythrose.*—Barium acetate monohydrate (14·89 g.), dissolved in water (43 c.c.), and ferric sulphate (7·31 g.), also dissolved in water (43 c.c.), were slowly added to water (1·42 l.) in equimolecular portions with stirring. The resulting solution was slowly boiled and calcium D-arabonate (123·40 g.) was slowly added to it. When the addition was complete the solution was boiled for 20 minutes and then filtered through a carbon pad and cooled to 40°; hydrogen peroxide (86·04 c.c., 100-vol.) was then added. The temperature rose to 60° and the solution became dark. After it had been again cooled to 40°, a second portion of hydrogen peroxide was added (86·04 c.c.) and the mixture was set aside overnight. Then it was filtered through a charcoal pad, and the filtrate was concentrated under diminished pressure to 160 c.c. Methyl alcohol (1075 c.c.) was added to it, and the solid which separated was removed by filtration. The residue was washed with methyl alcohol (215 c.c.) and the washings were added to the filtrate. Ether (645 c.c.) was added to the methyl-alcoholic extract, and after standing for 5 minutes, it was refiltered. Removal of the solvent from the filtrate by evaporation yielded a pale yellow syrup which set to a glass. The syrup was strongly reducing to Fehling's solution. Trituration with either methyl alcohol or glacial acetic acid did not induce crystallisation. The changes of rotation (*c*, 3·244 in water) were as follows:

Time, hours	—	0·166	0·65	2·50	3·63	23·38	49·23	73·23	97·23
$[\alpha]_D^{14·5°}$	−1·23°	−2·46°	−4·31°	−5·24°	−11·09°	−11·71°	−14·79°	−18·5°	−18·5°

Felton and Freudenberg (*loc. cit.*) give +30·5° after 2 days for the L-isomer; Wohl (*Ber.*, 1899, **32**, 3667) gives +32·7°; Weerman (*Rec. Trav. chim.*, 1917, **37**, 15) +23·6°; Deulofeu (*J.*, 1932, 1973) +22·1°; Ruff (*Ber.*, 1901, **34**, 1365) +21·5°. Further, for $[\alpha]_D$ for D-erythrose, Ruff (*Ber.*, 1899, **32**, 3672) gives −14·8° and Weerman (*Rec. Trav. chim.*, 1932, **37**, 16) −14·5°.

The syrup (yield 35 g., 46%) (Found: C, 39·6; H, 6·8. Calc. for $C_4H_8O_4$: C, 40·0; H, 6·66%) was shown to be D-erythrose by conversion into *iso*propylidene methylerythroside and into erythrosazone.

2 : 3-iso*Propylidene αβ-Methyl-*D-*erythroside.*—D-Erythrose syrup (4·51 g.), dry acetone (100 ml.), dry methyl alcohol (10 ml.) containing 0·2% sulphuric acid, and anhydrous copper sulphate (10 g.) were shaken together overnight. After filtration, the filtrate was neutralised with barium carbonate and then refiltered. Removal of the solvents by evaporation gave an oily residue, which distilled as a pale yellow liquid, b. p. 100° (bath temp.)/10 mm.; $[\alpha]_D^{14·5°}$ −55·5° (*c*, 0·054 in chloroform) (Found: OMe, 17·3.

Calc. for $C_8H_{14}O_4$: OMe, 17·8%) (2 : 3-*iso*propylidene methyl-L-erythroside has b. p. 45—50°/2 mm., $[\alpha]_D$ +57·4° in chloroform; Felton and Freudenberg, *loc. cit.*). This liquid (4·0 g., 61%) was non-reducing to Fehling's solution in the cold, but on heating for a few minutes it suddenly reduced the solution.

Hydrolysis of 2 : 3-iso*Propylidene αβ-Methyl-*D*-erythroside.*—This erythroside (1 g.) was dissolved in 0·1N-sulphuric acid, and the solution kept at room temperature for 3 days, the rotation then being constant. Glacial acetic acid (1·5 c.c.) was added, and the sulphate ions were removed by adding barium hydroxide solution. The solution, filtered through charcoal, was evaporated to dryness. There remained a pale yellow viscid syrup (0·58 g.) which was completely soluble in ethyl alcohol and strongly reduced Fehling's solution in the cold; $[\alpha]_D^{17°}$ −19·1° (equilibrium in 3 days) (*c*, 2·14 in water); cf. $[\alpha]_D^{14·5°}$ −18·5° for D-erythrose.

D-*Erythrose Phenylosazone.*—The foregoing syrup (0·5 g.) was dissolved in ethyl alcohol (5 c.c.) and to it was added a solution of phenylhydrazine (1·4 g.) in glacial acetic acid. The mixture was heated on a water-bath for 30 minutes and then poured into water. An oil separated, which was washed with water by decantation. After two days, the oil solidified, and was collected and recrystallised from ethyl alcohol, forming a yellow powder (0·35 g.), m. p. 160—162·5°; Felton and Freudenberg (*loc. cit.*) give m. p. 160—163°.

1 : 4 : 6-*Triacetyl* pseudo*Glucal.*—Triacetyl glucal (12·95 g.) was heated under reflux for 15 minutes with water (259 g.), and the solution concentrated under diminished pressure. The syrupy residue was taken up in ether, and the solution washed with sodium hydrogen carbonate solution and dried ($MgSO_4$). After distillation of the solvent, there were added to the syrupy residue (2·64 g.) freshly distilled acetic anhydride (60 c.c.) and fused sodium acetate (6 g.), and the mixture heated on a boiling water-bath for 3 hours, and then evaporated to dryness under diminished pressure. Ethyl alcohol was distilled twice over the residue, which was afterwards extracted with ether. The ethereal extract was dried ($MgSO_4$), and the solvents removed by distillation. The residue distilled as a pale yellow syrup (1·31 g.); b. p. 115—125°/0·01 mm., $[\alpha]_D^{18·5°}$ +66·8° (*c*, 0·898 in chloroform), $n_D^{19°}$ 1·4839 (Found: C, 52·7; H, 5·9. Calc. for $C_{12}H_{16}O_7$: C, 52·92; H, 5·91%); it decolorised bromine water. Bergmann *et al.* (*Annalen*, 1923, **434**, 79) give b. p. 150—165°/0·2—0·3 mm.

1 : 4 : 6-*Triacetyl* 2 : 3-*Dideoxyglucose* (with Dr. J. Staněk).—1 : 4 : 6-Triacetyl *pseudo*glucal (0·5 g.) was dissolved in absolute ether (50 c.c.) and after addition of palladium catalyst (on charcoal) the solution was hydrogenated under a slight pressure at room temperature. The catalyst was removed by filtration, and then the solvent by evaporation. The residual *triacetyl dideoxyglucose* was a colourless oil, which distilled as a colourless liquid, b. p. *ca.* 120—130°/0·01 mm. (bath temp.), $[\alpha]_D^{15°}$ +32·63° (*c*, 0·8587 in chloroform), $n_D^{15°}$ 1·4548 (Found: C, 52·6; H, 6·9. $C_{12}H_{18}O_7$ requires C, 52·55; H, 6·6%).

Ozonisation of 1 : 4 : 6-*Triacetyl* pseudo*Glucal.*—Triacetyl *pseudo*glucal (0·595 g.) was dissolved in glacial acetic acid (10 ml.) and a stream of oxygen–ozone was passed through the solution at 12°, until it no longer decolorised a solution of bromine in carbon tetrachloride. After dilution with ether (30 c.c.), zinc dust (10 g.) was added, and the mixture heated under reflux until the solution failed to colour starch–iodide paper. After filtration, the ether was removed by evaporation, and the acetic acid by distillation with alcohol. The residue, which was strongly reducing to Fehling's solution, was extracted with alcohol containing a few drops of water. Removal of the solvent from the extract gave 2 : 4-*diacetyl* D-*erythrose* as a colourless syrup (0·42 g., 95·4%). Trituration with ethyl alcohol and refrigeration did not induce crystallisation; $n_D^{16°}$ 1·4711, $[\alpha]_D^{17·5°}$ −20·9° (*c*, 0·572 in chloroform) (Found: C, 46·5; H, 5·5. $C_8H_{12}O_6$ requires C, 47·0; H, 5·8%).

Hydrolysis of 2 : 4-*Diacetyl* aldehydo-D-*Erythrose.*—The diacetyl compound (0·2 g.) and 0·05 N-hydrochloric acid were heated together under reflux for 5 hours. The solution was neutralised with barium carbonate, the filtered solution evaporated to dryness, and the residue extracted with ethyl alcohol. The extract was again evaporated to dryness, and the procedure repeated twice more. A pale yellow syrup (0·08 g.) remained which was strongly reducing to Fehling's solution; $[\alpha]_D^{17·5°}$ −19·7° (*c*, 2·47 in water; equilibrium value); phenylosazone, m. p. 160—163° alone or in admixture with D-erythrose phenylosazone.

1 : 2-iso*Propylidene Glucofuranose* 5 : 6-*Carbonate.*—Anhydrous glucose (16 g.) was suspended in freshly dried acetone (150 c.c.), and carbonyl chloride was passed through the suspension for 10 hours. After standing overnight, unchanged glucose (6·4 g.) was removed by filtration. The filtrate was neutralised with basic lead carbonate and then refiltered. The residue was washed with acetone, and the washings added to the filtrate. The volume of the solution was reduced to 10 c.c. and the solid which separated was collected and recrystallised from absolute ethyl alcohol, forming colourless needles (4·70 g.), m. p. 226° (sinters 215—216°), $[\alpha]_D^{20°}$ −37·4° (*c*, 0·560 in ethyl alcohol). Haworth and Porter (*loc. cit.*) give m. p. 223—224° (decomp.), $[\alpha]_{5780}^{20°}$ −36°.

3-*Methanesulphonyl* 1 : 2-iso*Propylidene Glucofuranose* 5 : 6-*Carbonate.*—The dry carbonate (above) (0·7812 g.) was dissolved in dry pyridine (10 c.c.), the solution cooled to 0°, and methanesulphonyl chloride (1·1 mols., 0·401 g.) slowly added. The mixture was left at room temperature overnight, and then poured into water and extracted with chloroform. The extract was dried ($CaCl_2$) and then evaporated to dryness. A syrup remained which crystallised on trituration with ethyl alcohol. Recrystallisation from ethyl alcohol yielded long colourless needles of the *methanesulphonyl* derivative (0·95 g., 92·2%); m. p. 136—137°, $[\alpha]_D^{18·5°}$ −22·1° (*c*, 0·452 in chloroform) (Found: C, 40·7; H, 5·0; S, 9·7. $C_{11}H_{18}O_9S$ requires C, 40·7; H, 4·9; S, 9·9%).

Glucofuranose 5 : 6-*Carbonate.*—1 : 2-*iso*Propylidene glucofuranose 5 : 6-carbonate (0·6 g.) was dissolved in ethyl alcohol (17 c.c.) containing concentrated hydrochloric acid (0·83 c.c.), and the solution heated at 70—75° for 40 minutes. On cooling, crystals separated; these were filtered off and shown to be unchanged material (0·3 g.). The filtrate was evaporated at 45—50° and water (30 c.c.) was gradually added so that the total volume did not fall below 17 c.c. After this treatment the solution was neutralised with silver carbonate and filtered. The filtrate was concentrated to dryness, and the residue extracted with hot methyl alcohol. The solvent was removed by evaporation, and the residue recrystallised from methyl alcohol in small crystals; m. p. 179°, $[\alpha]_D^{17°}$ +18·1° (*c*, 0·442 in water); yield 0·2 g., 68·3% based on the material undergoing change (Found: C, 40·9; H, 5·4. Calc. for $C_7H_{10}O_7$: C, 40·8; H, 4·9%). Haworth and Porter (*loc. cit.*) give m. p. 181°, $[\alpha]_{5780}^{20°}$ +18° in water.

1 : 2-*iso*Propylidene glucofuranose 5 : 6-carbonate (0·5 g.) with excess of barium hydroxide solution at 70° yields 1 : 2-*iso*propylidene glucofuranose, m. p. 158—159°, $[\alpha]_D^{15\cdot5°}$ −13·6° (*c*, 0·292 in water).

β-Methylglucofuranoside 5 : 6-Carbonate.—The foregoing carbonate (1 g.) was dissolved in methyl alcohol (25 c.c.) containing sulphuric acid (0·3 c.c.). After 5 hours at 45° the solution was exactly neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. The residue was dissolved in methyl alcohol, filtered, and ether added. The methylglucofuranoside 5 : 6-carbonate separated, and was filtered off and recrystallised from methyl alcohol–ether; m. p. 142—144°, $[\alpha]_D^{22°}$ −64° (*c*, 0·7 in water); yield 0·8 g. (Found: OMe, 14·7. Calc. for $C_8H_{12}O_7$: OMe, 14·1%). Haworth and Porter (*loc. cit.*) give m. p. 143—145°, $[\alpha]_{5770}^{22°}$ −66°.

Oxidation of β-Methylglucofuranoside 5 : 6-Carbonate with Lead Tetra-acetate.—The carbonate (0·8 g.) was dissolved in dry benzene (10 ml.), and lead tetra-acetate (2 g., 2 mols.) suspended in glacial acetic acid was added. The mixture was kept at 0° for 7 days and then poured into water. The product was extracted with light petroleum (b. p. 60—80°), and the extract washed with water, dried, and evaporated to dryness. A syrup remained, which was heated at 70° for 30 minutes with aqueous barium hydroxide solution. A white precipitate separated, and was removed by filtration. Carbon dioxide was passed through the filtrate, and the barium carbonate precipitated was filtered off. The filtrate was evaporated to dryness and yielded a pale yellow syrup which set to a glass (0·2 g.); $[\alpha]_D^{18°}$ −17·6° (equilibrium in 72 hours) (*c*, 1·45 in water). The syrup yielded a phenylosazone, m. p. 160—162°, identical with D-erythrose phenylosazone.

1-*Nitro*-D-erythro-3 : 4 : 5-*triacetoxypent*-1-*ene*.—D-Erythrose (5·40 g.) was suspended in dry methyl alcohol (50 ml.). To it was added nitromethane (33·7 c.c.), followed by methyl alcohol (50 c.c.) containing sodium (1·42 g.). The mixture was shaken for 24 hours and then diluted with ether (40 c.c.). A solid separated and was filtered off. It was dissolved in water and passed through a Zeocarb-resin column, in order to remove sodium ions. The effluent was evaporated to dryness and yielded a syrupy residue (*A*). To the original filtrate obtained from the above solid, glacial acetic acid (3 c.c.) was added, and the whole evaporated to dryness. The residue was dissolved in water, and the solution passed through a Zeocarb-resin column, to remove sodium ions. The effluent was concentrated to dryness and thoroughly dried in a vacuum desiccator over phosphoric oxide, and the residue was added to (*A*). Redistilled acetic anhydride (excess) and concentrated sulphuric acid (1 drop) were added to this, and the mixture was heated under reflux for one hour. Excess of acetic anhydride was removed by distillation. Dry benzene (150 c.c.) and sodium hydrogen carbonate (15·0 g.) were added to the residue, and the mixture was boiled for 2·5 hours. Filtration and evaporation of the benzene from the filtrate afforded the syrupy *nitropentene* (8·48 g., 65·2%) as a yellow oil; $n_D^{18°}$ 1·4553, $[\alpha]_D^{20°}$ −8·6° (*c*, 1·156 in ethyl alcohol) (Found: C, 45·6; H, 5·15; N, 4·8. $C_{11}H_{15}O_8N$ requires C, 45·6; H, 5·2; N, 4·8%). On attempted distillation, it underwent decomposition at 160—170° (bath temp.)/0·005 mm. After standing for 3 months, the syrup commenced to crystallise. Recrystallisation from alcohol–water gave crystals, m. p. 116—118° (Found: C, 45·2; H, 5·4; N, 4·3%). The material decolorised bromine in alcohol and potassium permanganate solution.

2-*Deoxy*-D-*ribose Anilide*.—The acetylated nitropentene (1·28 g.) was dissolved in dry methyl alcohol (25 c.c.) and palladium-black (0·2 g.) was added. The solution was hydrogenated at room temperature until no further absorption of hydrogen occurred. After filtration, the solution was evaporated to dryness and a thick syrup remained. To this IN-sodium hydroxide (8 c.c.) was added and the mixture was kept at room temperature for 1 hour. It was then added to a stirred mixture of water (1·5 c.c.) and concentrated sulphuric acid (1·05 c.c.) at room temperature. (At this stage the solution gave a purple Dische reaction.) The solution was neutralised with barium carbonate, stirred with charcoal, filtered, and the filtrate evaporated to dryness. The residue strongly reduced Fehling's solution, and gave a blue Dische colour. It was dissolved in water (2 c.c.) and aniline (0·2 c.c.) was added, together with sufficient ethyl alcohol to make the solution homogeneous. The solution was kept at 0° for 5 days. The ethyl alcohol was removed by evaporation, and on addition of water (5 c.c.) to the residue, a solid separated. This was collected by filtration, and washed with ice-cold ethyl alcohol, but remained faintly yellow; m. p. 175—176°, mixed m. p. with 2-deoxy-D-ribose anilide, 173—174°; $[\alpha]_D$ +20·5° (equilibrium) (*c*, 1·05 in ethyl alcohol); yield 12 mg.

2-*Deoxy*-D-*ribose*.—2-Deoxy-D-ribose anilide (0·125 g.) and 0·5% oxalic acid solution in water (10 c.c.) were heated at 80° for 1 hour. The solution was extracted once with ether to remove aniline, and the aqueous layer was heated under reflux with calcium carbonate. After filtration, the filtrate was evaporated to dryness. The residue was extracted with hot ethyl acetate, and the extract was filtered after standing for some hours. The ethyl acetate was removed by evaporation, and the residue was taken up in dry *iso*propyl alcohol and set aside. Crystals of 2-deoxy-D-ribose separated (0·045 g., 23·3%), m. p. 87—90°, $[\alpha]_D^{22°}$ −55·2° (equilib.) in water (*c*, 0·90). Levene and Mori (*J. Biol. Chem.*, 1929, **83**, 803) give m. p. 80°.

Anilination of 2-Deoxy-D-ribose.—A suspension of 2-deoxy-D-ribose (0·14 g.) in absolute ethyl alcohol (5 c.c.) was heated under reflux with an alcoholic solution (5 c.c.) of aniline (0·11 g.) for 3 hours, by which time the solid had dissolved. Concentration of the solution by distillation under reduced pressure gave crystals of 2-deoxy-D-ribose anilide (0·19 g., 90%), m. p. 174—175°, $[\alpha]_D^{23°}$ +19·5° (equilibrium) (*c*, 1·05 in ethyl alcohol) (Found: C, 63·25; H, 7·3; N, 6·30. Calc. for $C_{11}H_{15}O_3N$: C, 63·1; H, 7·2; N, 6·7%). Kent, Stacey, and Wiggins (this vol., p. 1232) give m. p. 165—166°.

The microanalyses were carried out by Dr. W. T. Chambers. One of us (W. G. O.) thanks the British Rubber Producers' Research Association for financial assistance which enabled him to take part in this investigation.

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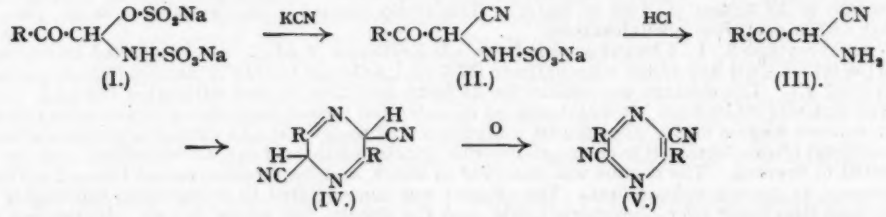
289. *Pyrazine Derivatives. Part X. 2 : 5-Disubstituted 3 : 6-Dicyanopyrazines.*

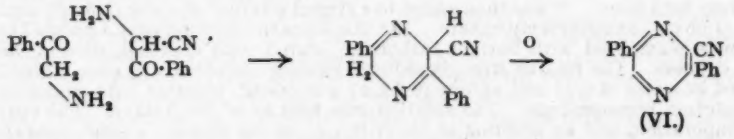
By Ezra Golombok and F. S. Spring.

The Gastaldi method for the preparation of 2 : 5-disubstituted 3 : 6-dicyanopyrazines is discussed, and some reactions of 3 : 6-dicyano-2 : 5-dimethylpyrazine are described.

The method of Gastaldi (*Gazzetta*, 1921, **51**, I, 233) has been used by Sharp and Spring (*J.*, 1948, 1862) for the preparation of 3 : 6-dicyano-2 : 5-dialkylpyrazines. In this reaction the bisulphite derivative of an oximino-ketone is treated successively with potassium cyanide and hydrochloric acid; according to Gastaldi, the bisulphite derivative (I) reacts with potassium cyanide to give the compound (II), acid hydrolysis of which yields an α-amino-α-cyano-ketone (III). Self-



condensation of (III) gives a dicyanodihydropyrazine (IV) which is oxidised by air to the dicyanopyrazine (V). This reaction scheme is improbable, since it assumes the self-condensation of the amino-cyano-ketone (III) in a strongly acid medium. α-Amino-ketones are stable in acid solution, the typical pyrazine condensation occurring in either a neutral or alkaline medium. When the reaction is applied to the bisulphite derivative of oximinoacetophenone, a mixture of 3 : 6-dicyano-2 : 5-diphenylpyrazine (V; R = Ph) and 3-cyano-2 : 5-diphenylpyrazine (VI) is obtained. Gastaldi attributes formation of the latter compound to the formation of ω-aminoacetophenone by the decomposition of part of the intermediate amino-cyano-ketone. Condensation of ω-aminoacetophenone with the amino-cyano-ketone is assumed to give 3-cyano-2 : 5-diphenyldihydropyrazine, oxidation of which yields 3-cyano-2 : 5-diphenylpyrazine :

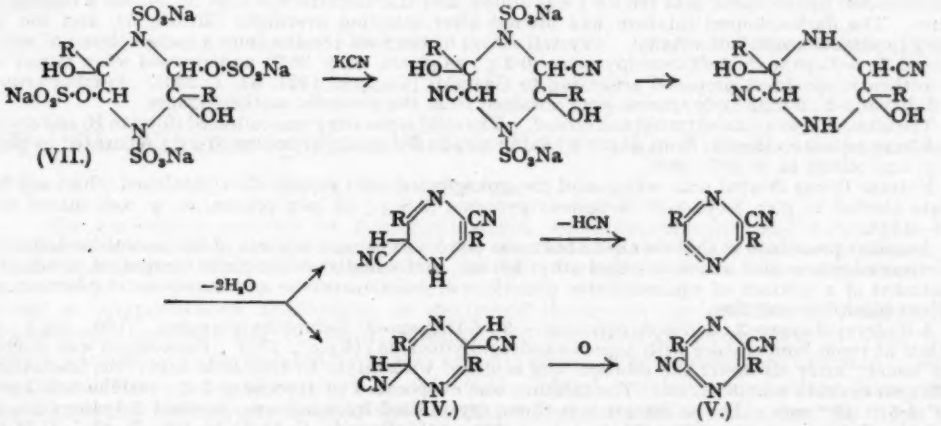


These assumptions are not only improbable, but also unnecessary. Thus we find that the addition of aminoacetone hydrochloride to the Gastaldi reaction mixture from oximinoacetone bisulphite derivative at the commencement of the acid hydrolysis stage does not lead to the formation of 3-cyano-2 : 5-dimethylpyrazine. The formation of 3-cyano-2 : 5-diphenylpyrazine from oximinoacetophenone is almost certainly to be attributed to an alternative method for the aromatisation of the dihydro-intermediate (IV) involving loss of hydrogen cyanide. This view accords with numerous similar observations of alternative means by which dihydropyrazine derivatives change into the aromatic form. Thus, treatment of alanine anhydride with phosphoryl chloride gives 3-chloro-2 : 5-dimethylpyrazine in addition to 3 : 6-dichloro-2 : 5-dimethylpyrazine (Baxter and Spring, *J.*, 1947, 1179). Again, treatment of DL-phenylglycine anhydride with phosphoryl chloride yields 3-hydroxy-2 : 5-diphenylpyrazine in addition to 3 : 6-dichloro-2 : 5-diphenylpyrazine (Gallagher, Newbold, Spring and Woods, this vol., p. 910).

Concerning the mechanisms of the principal Gastaldi reaction, it seems to us more plausible to ascribe the dimeric formula (VII) to the intermediate bisulphite derivatives, the subsequent stages in the reaction being as shown on p. 1365.

In support of this view we find that treatment of a mixture of the bisulphite derivatives of oximinoacetone and oximinomethyl ethyl ketone with potassium cyanide, and then with hydrochloric acid, gives a mixture of products from which only 3 : 6-dicyano-2 : 5-dimethylpyrazine and 3 : 6-dicyano-2 : 5-diethylpyrazine could be obtained; 3 : 6-dicyano-2-methyl-5-ethylpyrazine was not isolated. Similarly, when a mixture of the bisulphite derivatives of

oximinoacetophenone and oximinoacetone was subjected to the Gastaldi reaction, the formation of 3 : 6-dicyano-2-phenyl-5-methylpyrazine was not observed, 3 : 6-dicyano-2 : 5-dimethyl-



pyrazine, 3 : 6-dicyano-2 : 5-diphenylpyrazine, and 3-cyano-2 : 5-diphenylpyrazine being the only products isolated. Although these observations appear to favour the cyclic dimeric structure (VII) rather than the simpler structure (I) for the bisulphite derivatives of oximino-ketones, this argument must be used with caution, particularly in view of the fact that, when a mixture of oximinoacetone and oximinoacetophenone was treated with sodium bisulphite solution, and the mixed bisulphite derivative was subjected to the Gastaldi reaction, the formation of 3 : 6-dicyano-2-phenyl-5-methylpyrazine was not observed, only 3 : 6-dicyano-2 : 5-dimethylpyrazine, 3 : 6-dicyano-2 : 5-diphenylpyrazine, and 3-cyano-2 : 5-diphenylpyrazine being isolated. A molecular-weight determination for the bisulphite derivative of an oximino-ketone would help to differentiate between the structures (I) and (VII), but so far it has been impossible to free these compounds from sodium bisulphite.

Some further reactions of 3 : 6-dicyano-2 : 5-dimethylpyrazine have been studied. Treatment with dilute sodium hydroxide solution at room temperature gives 3-*hydroxy*-6-*cyano*-2 : 5-*dimethylpyrazine*,* and similar treatment of 3 : 6-dicyano-2 : 5-diethylpyrazine yields 3-*hydroxy*-6-*cyano*-2 : 5-*diethylpyrazine*. When heated under reflux with concentrated alkali, these hydroxy-cyano-compounds are hydrolysed to the previously described 3-hydroxy-2 : 5-dialkylpyrazine-6-carboxylic acids (Sharp and Spring, *J.*, 1948, 1862). With boiling phosphoryl chloride, 3-hydroxy-6-cyano-2 : 5-dimethylpyrazine yields 3-*chloro*-6-*cyano*-2 : 5-*dimethylpyrazine*.

Treatment of 3 : 6-dicyano-2 : 5-dimethylpyrazine with sodium ethoxide gives 3-*cyano*-6-*ethoxy*-2 : 5-*dimethylpyrazine* which, on hydrolysis with mineral acid, is converted into 3-hydroxy-6-cyano-2 : 5-dimethylpyrazine. Various peroxidations of 2-substituted pyrazine derivatives have been described by Baxter, Newbold and Spring (*J.*, 1948, 1859). Although these oxidations were undertaken with the object of preparing a cyclic hydroxamic acid of pyrazine, they led, in general, to the formation of 3-substituted pyrazine 1-oxides and not to the required 2-substituted pyrazine 1-oxide. The oxidation of 3-cyano-6-ethoxy-2 : 5-dimethylpyrazine was investigated since, if oxidation occurs at either the 1- or 4-positions, suitable treatment of the product may give rise to a cyclic hydroxamic acid. When treated with hydrogen peroxide in acetic acid, 3-cyano-6-ethoxy-2 : 5-dimethylpyrazine gave a *mono-oxide* which liberates iodine from acidified potassium iodide. If this is the 1-oxide, hydrolysis with hydrochloric acid would be expected to give a cyclic hydroxamic acid; however, it led to rupture of the ring system, hydrogen cyanide being evolved and ammonium chloride produced. A similar decomposition occurred when the oxide was treated with alkali.

Experimental.

3 : 6-*Dicyano*-2 : 5-*dimethylpyrazine*, 3 : 6-*Dicyano*-2 : 5-*diphenylpyrazine*, *and* 3-*Cyano*-2 : 5-*diphenylpyrazine*.—A mixture of the crude bisulphite compounds, individually prepared from oximinoacetone

* This compound was first prepared by Dr. R. A. Raphael by hydrolysis of the dinitrile with 15% methanolic potassium hydroxide (private communication).

(9 g.) and oximinoacetophenone (15 g.), was shaken with water (80 c.c.), and potassium cyanide (45 g.) was added in portions. When dissolution was complete, the mixture was kept at room temperature for 3 hours. Hydrochloric acid (20%; 40 c.c.) was added, and the mixture kept at 55° for 3 hours. Concentrated hydrochloric acid (80 c.c.) was added, and the mixture was kept at 55° for a further four hours. The dark-coloured mixture was filtered after standing overnight (filtrate A), and the solid (3·4 g.) extracted with hot ethanol. Crystallisation of the final residue from a large volume of ethanol yielded 3 : 6-dicyano-2 : 5-diphenylpyrazine (0·3 g.) as plates, m. p. 203°, undepressed when mixed with an authentic specimen prepared according to Gastaldi (*Gazzetta*, 1921, **51**, I, 233). Further crops of 3 : 6-dicyano-2 : 5-diphenylpyrazine were obtained from the alcoholic mother-liquors.

The filtrate A was concentrated and cooled. The solid separating was collected (filtrate B) and crystallised from aqueous ethanol, from which 3 : 6-dicyano-2 : 5-dimethylpyrazine (0·7 g.) separated as plates, m. p. and mixed m. p. 207—208°.

Filtrate B was diluted with water, and the precipitated solid repeatedly crystallised (charcoal) from dilute alcohol to give 3-cyano-2 : 5-diphenylpyrazine (0·8 g.) as long prisms, m. p. and mixed m. p. 120—121°.

A similar procedure to that detailed above was employed using a mixture of the bisulphite derivatives of oximinoacetone and oximinomethyl ethyl ketone, and using the bisulphite compound obtained by treatment of a mixture of equimolecular quantities of oximinoacetone and oximinoacetophenone with sodium bisulphite solution.

3-Hydroxy-6-cyano-2 : 5-dimethylpyrazine.—3 : 6-Dicyano-2 : 5-dimethylpyrazine (100 mg.) was shaken at room temperature with aqueous sodium hydroxide (10 c.c.; 5%). Dissolution was complete in 4 hours; after six hours the solution was acidified with dilute hydrochloric acid; the liberation of hydrogen cyanide was detected. The mixture was evaporated to dryness and the residue sublimed at 140°/5·8 × 10^{-4} mm. The sublimate was thrice crystallised from benzene, to yield *3-hydroxy-6-cyano-2 : 5-dimethylpyrazine* as needles (50 mg.), m. p. 220—221° (Found: C, 56·2; H, 4·8; N, 27·7. $C_7H_7ON_3$ requires C, 56·4; H, 4·7; N, 28·2%).

A solution of 3-hydroxy-6-cyano-2 : 5-dimethylpyrazine (25 mg.) in 15% aqueous potassium hydroxide (5 c.c.) was heated under reflux for 10 hours; ammonia was evolved. The solution was acidified with concentrated hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with boiling ethanol, the extract evaporated to dryness, and the residue crystallised from water, to give 3-hydroxy-2 : 5-dimethylpyrazine-6-carboxylic acid as needles, m. p. 265° undepressed when mixed with the specimen described by Sharp and Spring (*J.*, 1948, 1862).

3-Hydroxy-6-cyano-2 : 5-diethylpyrazine.—Treatment of 3 : 6-dicyano-2 : 5-diethylpyrazine with 5% potassium hydroxide solution at room temperature, as described above, gave *3-hydroxy-6-cyano-2 : 5-diethylpyrazine* which separated from benzene as needles, m. p. 129—130° (yield, 40%) (Found: C, 60·4; H, 6·3; N, 23·3. $C_9H_{11}ON_3$ requires C, 61·0; H, 6·2; N, 23·7%). Hydrolysis of 3-hydroxy-6-cyano-2 : 5-diethylpyrazine with boiling 15% aqueous potassium hydroxide gives 3-hydroxy-2 : 5-diethylpyrazine-6-carboxylic acid as prisms (from benzene), m. p. 164°, undepressed when mixed with the specimen described by Sharp and Spring (*loc. cit.*).

3-Cyano-6-ethoxy-2 : 5-dimethylpyrazine.—3 : 6-Dicyano-2 : 5-dimethylpyrazine (2·0 g.) was shaken for 10 hours at room temperature with a solution of sodium ethoxide in ethanol (from 1 g. of sodium and 25 c.c. of ethanol). The mixture was evaporated under reduced pressure, and the residue dissolved in a little water and acidified with hydrochloric acid. The precipitated solid was collected, washed with water, and dried. Sublimation of this solid at 80°/1·5 × 10^{-4} mm. gave *3-cyano-6-ethoxy-2 : 5-dimethylpyrazine*, which separated from aqueous ethanol as needles (1·5 g.), m. p. 61° (Found: C, 60·7; H, 6·3; N, 23·9. $C_9H_{11}ON_3$ requires C, 61·0; H, 6·2; N, 23·7%). After removal of 3-cyano-6-ethoxy-2 : 5-dimethylpyrazine, the temperature was raised. At 180°/1·5 × 10^{-4} mm., a second sublimate was obtained, which separated from benzene as needles, m. p. 219°, undepressed when mixed with 3-hydroxy-6-cyano-2 : 5-dimethylpyrazine (m. p. 220—221°).

3-Cyano-6-ethoxy-2 : 5-dimethylpyrazine (40 mg.) was heated under reflux for 2 hours with 4N-hydrochloric acid (5 c.c.). The solution was evaporated to dryness, and the residue sublimed at 180°/1·5 × 10^{-4} mm. The sublimate was crystallised from benzene to yield 3-hydroxy-6-cyano-2 : 5-dimethylpyrazine as needles, m. p. 219°, undepressed when mixed with the specimen described above.

3-Cyano-6-ethoxy-2 : 5-dimethylpyrazine (500 mg.) was heated under reflux for 10 hours with 50% sulphuric acid (20 c.c.); carbon dioxide was evolved during the reaction. The cold solution was neutralised with ammonia and extracted with ether. The dried (Na_2SO_4) extract was evaporated, and the residue crystallised from benzene (charcoal), to give 3-hydroxy-2 : 5-dimethylpyrazine as needles, m. p. 208—209°, undepressed when mixed with an authentic specimen.

3-Cyano-6-ethoxy-2 : 5-dimethylpyrazine (0·7 g.) was boiled under reflux for 10 hours with 5N-potassium hydroxide (15 c.c.); ammonia was evolved. The cold solution was acidified with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with boiling alcohol, the extract concentrated, and the solid separating recrystallised twice from alcohol, from which 3-hydroxy-2 : 5-dimethylpyrazine-6-carboxylic acid (0·45 g.) separated as needles, m. p. 265° (decomp.), undepressed when mixed with the specimen described above.

3-Cyano-6-ethoxy-2 : 5-dimethylpyrazine oxide.—A solution of 3-cyano-6-ethoxy-2 : 5-dimethylpyrazine (0·32 g.) in acetic acid (3 c.c.) was treated with hydrogen peroxide (100 vol.; 3 c.c.) and kept at 55° for 20 hours. The cold solution was poured into water, and the solid was collected, washed with water and dried. Crystallisation from light petroleum (b. p. 40—60°) gave *3-cyano-6-ethoxy-2 : 5-dimethylpyrazine oxide* (0·18 g.) as needles, m. p. 104° (Found: C, 56·0; H, 5·7; N, 21·5. $C_9H_{11}O_2N_3$ requires C, 56·0; H, 5·7; N, 21·8%). The oxide liberates iodine from an acidified potassium iodide solution.

3-Chloro-6-cyano-2 : 5-dimethylpyrazine.—3-Hydroxy-6-cyano-2 : 5-dimethylpyrazine (200 mg.) was treated with phosphoryl chloride (7 c.c.), and the mixture heated under reflux for 30 minutes and then evaporated under reduced pressure. The residual oil was triturated with a little ethanol until crystallisation occurred. Recrystallisation from aqueous ethanol gave *3-chloro-6-cyano-2 : 5-dimethylpyrazine* as

plates (0·75 g.), m. p. 79—80° (Found: C, 50·4; H, 3·9; N, 24·6; Cl, 20·9. $C_7H_4N_2Cl$ requires C, 50·15; H, 3·6; N, 25·1; Cl, 21·2%).

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THE ROYAL TECHNICAL COLLEGE, GLASGOW. [*Received, November 12th,* 1948.]

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290. *The Nitration of Some Simple Heterocyclic Nitrogen Compounds.*

By K. SCHOFIELD and T. SWAIN.

The qualitative nitration of 4-methylcinnoline, 4-hydroxyquinoline, and 1-methyl-4-cinnolone is described, and previous work on the nitration of quinazoline is confirmed. These and other qualitative results are discussed in the light of current views on modes of nitration.

BEFORE a comprehensive application of electronic theory to the problem of substitution in heterocyclic systems can be made, especially in quantitative terms such as have been developed for benzene derivatives by Ingold and his school, it is necessary to examine to what extent qualitative regularities exist in this field. Many isolated observations have been recorded relating to such problems as the nitration of heterocyclic systems, but the stress has usually been on the synthesis of new compounds, and not on the comparative chemistry of different heterocyclic systems, even in the qualitative way which concerns us here. This being so, we have examined the nitration of some simple compounds of the triple series, quinolines, quinazolines, cinnolines. Some of the results have already been outlined (Schofield and Swain, *Nature*, 1948, **161**, 690), and in the present communication we supply the essential details and some extensions of the work.

Before our work was complete, Elderfield and his collaborators (*J. Org. Chem.*, 1947, **12**, 405) reported that 6-nitroquinazoline was the main product (56%) of nitration of quinazoline in sulphuric acid; our experiments confirm this. Before the work of Elderfield and his associates (*loc. cit.*) the only practicable route to quinazoline derivatives unsubstituted in the hetero-ring was that of Riedel (G.P. 174941; cf. Bogert and McColm, *J. Amer. Chem. Soc.*, 1927, **49**, 2650, and Reynolds and Robinson, *J.*, 1936, 196). By this method 5-chloro-2-nitrobenzaldehyde gave *5-chloro-2-nitrobenzylidenebisformamide* and thence, by reduction, *6-chloroquinazoline* in high yield. The same chloro-compound was obtained by a Sandmeyer reaction with the 6-aminoquinazoline resulting from the reduction of the major nitration product of quinazoline (see above). No homogeneous product could be isolated when quinazoline was nitrated with lithium nitrate (cf. 7-nitroquinoline; Bacharach, *Rec. Trav. chim.*, 1933, **52**, 413).

According to most of the available analogies, 4-methylcinnoline should nitrate in the same sense as cinnoline itself. Thus, quinoline (see, among others, Curd, Graham, Richardson, and Rose, *J.*, 1947, 1613) and quinaldine (Doebner and von Miller, *Ber.*, 1884, **17**, 1700; Decker and Remfrey, *Ber.*, 1905, **38**, 2773; Hammick, *J.*, 1926, 1302) give mixtures of the 5- and 8-derivatives, the latter predominating slightly, whilst from lepidine the major product is 8-nitrolepidine (Busch and Koenigs, *Ber.*, 1890, **23**, 2687; Johnson and Hamilton, *J. Amer. Chem. Soc.*, 1941, **63**, 2864) and a minor product, possibly the 5-isomer, has also been isolated (Buchman *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 380). The 4-methyl group thus appears to exert a steric effect, and this weight of analogy led us to expect 4-methylcinnoline to yield mainly 8-nitro-4-methylcinnoline. Thus, we nitrated 4-methylcinnoline as a prelude to cinnoline itself; under conditions similar to those used for lepidine (Johnson and Hamilton, *loc. cit.*) the only product so far isolated (50%) is *8-nitro-4-methylcinnoline*; this was reduced to the *amine* and converted thence into *8-chloro-4-methylcinnoline*, m. p. 126—127°; this last compound depressed the melting point of 6-chloro-, m. p. 136—137°, and 7-chloro-4-methylcinnoline, m. p. 119—120° (Atkinson and Simpson, *J.*, 1947, 808), and its structure was proved by synthesis. 3-Chloro-2-aminoacetophenone (Simpson *et al.*, *J.*, 1945, 646) with methylmagnesium iodide gave an oily compound (the carbinol or a mixture thereof with the olefin), yielding, on dehydration, the oily olefin, which was characterised as *2-(3′-chloro-2′-acetamidophenyl)prop-1-ene* and converted by diazotisation into 8-chloro-4-methylcinnoline identical with the compound described above.

Attempted nitration of 4-methylquinazoline has not so far yielded homogeneous material under a variety of conditions. This problem is still being examined. 4-Methylquinazoline was originally prepared by hydrolysis and decarboxylation of 4-methylquinazoline-2-carboxyamide

(Bogert and Nabenhauer, *J. Amer. Chem. Soc.*, 1924, **46**, 1932). We now find that treatment of *o*-formamidoacetophenone (Camps, *Ber.*, 1901, **34**, 2703; contrast Bischler, *Ber.*, 1893, **26**, 1352) with ammonia under pressure gives 4-methylquinazoline in good yield.

The nitration of 4-hydroxyquinoline in sulphuric acid was recently stated to give about 50% of 3-nitro-4-hydroxyquinoline (Mosher *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 303). This was unexpected in view of the behaviour of the closely related 4-hydroxyquinazoline (Bogert and Geiger, *J. Amer. Chem. Soc.*, 1912, **34**, 524; Morley and Simpson, *J.*, 1948, 360) and 4-hydroxycinnoline (Schofield and Simpson, *J.*, 1945, 512; Simpson, *J.*, 1947, 237). In fact, the compound, m. p. 144—145°, described by Mosher *et al.* as 4-chloro-3-nitroquinoline, strongly depresses the melting point of authentic 4-chloro-3-nitroquinoline, m. p. 121—122° (Bachman *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 365), and the two compounds differ considerably in stability: 4-chloro-3-nitroquinoline is very readily hydrolysed, *e.g.*, during attempted crystallisation from methanol, whilst the chloro-compound, m. p. 144—145°, survives such crystallisation. The derived 4-phenoxy-compounds were also different, *3-nitro-4-phenoxyquinoline*, m. p. 108—109°, strongly depressing the melting point (117—118°) of the phenoxy-derivative of the nitration product. On the other hand, 4-chloro-6-nitroquinoline, m. p. 144—145° (Riegel *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 1264, 1267), and *6-nitro-4-phenoxyquinoline*, m. p. 117—118°, were identical with the corresponding derivatives of Mosher's nitration product.

The nitrations discussed above, and those of 4-hydroxyquinazoline and 4-hydroxycinnoline mentioned, were effected in sulphuric acid. Under similar conditions 4-hydroxyquinaldine also yields its 6-nitro-derivative (Kermack and Weatherhead, *J.*, 1939, 563), but, when the nitration is conducted in nitric acid at about 100°, the product is 3-nitro-4-hydroxyquinaldine (Conrad and Limpach, *Ber.*, 1887, **20**, 950; Halcrow and Kermack, *J.*, 1945, 415). Accordingly, we nitrated 4-hydroxyquinoline under the latter conditions, and from the total nitration product (obtained in 62% yield) the only homogeneous compound which we could characterise was 3-nitro-4-hydroxyquinoline (comprising 65% of the nitration product), isolated as its 4-chloro- and 4-phenoxy-derivatives.

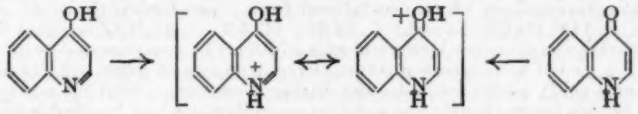
The qualitative evidence now available permits the distinguishing of two groups of compound among quinolines, quinazolines, and cinnolines, unsubstituted in the benz-ring, with regard to the direction of nitration:

(1*a*) α- and γ-Hydroxy-compounds, which are nitrated in sulphuric acid at C_6 or C_8 [4-hydroxyquinoline, 4-hydroxyquinazoline, 4-hydroxycinnoline, 4-hydroxyquinaldine, 2-hydroxylepidine (Balaban, *J.*, 1930, 2346), carbostyril(Friedlander and Lazarus, *Annalen*, 1885, **229**, 233; Decker, *J. pr. Chem.*, 1901, **64**, 85)]. (1*b*) Similar compounds, which are nitrated in the absence of sulphuric acid at the 3-position [4-hydroxyquinoline, 4-hydroxyquinaldine, 2 : 4-dihydroxyquinoline (Gabriel, *Ber.*, 1918, **51**, 1500. But see Ashley, Perkin and Robinson, *J.*, 1930, 382)].

(2*a*) Unsubstituted heterocyclic compounds, or those with one α- or γ-methyl or -chloro-group, which are nitrated at C_5 and/or C_8 by mixed acids [quinoline, *iso*quinoline (Claus and Hoffmann, *J. pr. Chem.*, 1893, ii, **47**, 253; Fortner, *Monatsh.*, 1893, **14**, 146; Le Fèvre and Le Fèvre, *J.*, 1935, 1470; Andersag, *Chem. Zentr.*, 1934, I, 3595), quinaldine, lepidine, 4-methylcinnoline, 2-chloroquinoline (Deinet and Lutz, *J. Amer. Chem. Soc.*, 1946, **68**, 1325), 3-chloroquinoline (Baker *et al.*, *ibid.*, p. 1532), 4-chloroquinoline (Mosher *et al.*, *loc. cit.*)]. (2*b*) Heterocyclic compounds with both α- and γ-positions substituted either by methyl or chloro-groups, or by one each of these, which are nitrated most readily at C_8 and somewhat less readily at C_5, but also to some extent at C_6 [2 : 4-dimethylquinoline (Price, Velzen and Guthrie, *J. Org. Chem.*, 1947, **12**, 203; Vaughan, *J. Amer. Chem. Soc.*, 1948, **70**, 2294), 2-chlorolepidine (Johnson and Hamilton, *loc. cit.*; Krahler and Burger, *J. Amer. Chem. Soc.*, 1942, **64**, 2417), 4-chloroquinaldine (Halcrow and Kermack, *loc. cit.*)].

In discussing these facts it is essential to decide precisely what entity is being nitrated in any case and also what is the true nitrating agent. With regard to the facts under (1*a*) there can be no doubt that the nitrating agent here is the nitronium ion NO_2^+ (Bennett, Brand, and Williams, *J.*, 1946, 869 *et seq.*; Hughes, Ingold, and Reed, *Nature*, 1946, **158**, 448; Halberstadt, Hughes, and Ingold, *ibid.*, p. 514; Bunton, Hughes, Minkoff, and Reed, *ibid.*, p. 514; Westheimer and Kharasch, *J. Amer. Chem. Soc.*, 1946, **68**, 1871). As to the nature of the molecule being nitrated it might seem that the old problem of the tautomerism of compounds of this type would be a complication, but, taking 4-hydroxyquinoline as an example, it is probable that protonisation of either (I) or (II) occurs, giving the resonance hybrid (III ⟷ IV), and it is presumably the hybrid which is nitrated under these circumstances. In (III ⟷ IV),

deactivation (with respect to an electrophilic reagent) occurs at C_5 and C_7, so that nitration is to be expected at C_6 and C_8. From another point of view the structure (IV) is seen to bear formal resemblance to protonated *o*-acetamidoacetophenone (V), which is known to give 5- and 3-nitro-2-acetamidoacetophenone when nitrated in sulphuric acid (Simpson *et al.*, *loc. cit.*), analogous to 6- and 8-nitro-4-hydroxyquinoline. Similar considerations apply to other compounds mentioned under (1*a*). In this connection we thought it of interest to nitrate 1-methyl-4-cinnolone (Schofield and Simpson, *loc. cit.*), in which the *p*-quinonoid structure (VI), similar to (IV), would be expected to exert a controlling influence; 8-nitro-1-methyl-4-cinnolone was the sole product (74%), identical with a specimen prepared from 8-nitro-4-hydroxycinnoline (Schofield



(I.) (III.) (IV.) (II.)

(V.) (VI.)

and Theobald, unpublished). As to the reactions under (1*b*), it seems likely that nitric acid, although weaker than sulphuric acid (Hammett, " Physical Organic Chemistry," Chapter IX, New York, 1940), will still be strong enough to protonise the hydroxy-heterocyclic compounds (Bunton, Hughes, Minkoff, and Reed, *loc. cit.*, have made a similar point with regard to phenols), so that the different mode of substitution must be due to the substituting reagent. Nitration in this medium will still involve an electrophilic reagent (either nitronium or nitracidium ions; Halberstadt, Hughes, and Ingold, *loc. cit.*) which would again be expected to nitrate the molecule at C_6 or C_8 (with carbostyril ethers, 1-methyl-2-quinolone, etc., where nitration proceeds with relative ease, this is actually the case; Kaufmann and de Petherd, *Ber.*, 1917, **50**, 336), but the effect of nitrous acid may be important. With phenols in particular, the intervention of the latter can reverse completely the proportions of *o*- and *p*-isomers formed (Bunton *et al.*, *loc. cit.*). In connexion with this category of compounds it is noteworthy that 4-hydroxycinnoline yields 6-nitro- and a little 8-nitro-4-hydroxycinnoline when nitrated in sulphuric acid (Schofield and Simpson; Simpson, *locc. cit.*), but that in nitric acid at 50°, whilst the 6-nitro-compound is still the main product, an unidentified isomer is also produced in small amount. It is possible that the compound, which may be 3-nitro-4-hydroxycinnoline [it is not the 5- or 7-nitro-compound (Schofield and Theobald, unpublished)], would be formed in higher yield at higher temperatures; we are examining this point.

With regard to compounds grouped under (2) we have pointed out that the simple resonance picture is inadequate (Schofield and Swain, *loc. cit.*), as is also the semi-quantitative model (Longuet-Higgins and Coulson, *Trans. Faraday Soc.*, 1947, **43**, 87). Recently, approximate calculations for quinazoline and cinnoline have appeared (Pullman, *Revue Scientifique*, 1948, **86**, 219) and it is interesting to notice that in the first case these lead us to expect electrophilic attack at C_6. With cinnoline the differences between the various positions in the benz-ring are small, and prediction would be difficult. It is becoming increasingly clear that the relative stabilities of the quinonoid transition complexes formed in these reactions may outweigh the initial distribution of electronic charge in determining the position of substitution (Waters, *J.*, 1948, 727). Quinazoline assumes special interest in this light, for perhaps here the *amphi*-quinonoid (C_2–C_6) form of the activated complex has particular importance, as is presumably the case with the compounds grouped under (2*b*). Waters (*loc. cit.*) stressed the importance of the oxidation–reduction potentials of relevant quinones for the present problem, and such measurements in the quinoline and *iso*quinoline fields support this view (Fieser and Martin, *J. Amer. Chem. Soc.*, 1935, **57**, 1840). We hope to make similar determinations in the quinazoline and cinnoline series.

Experimental.

(M. p.s are uncorrected.)

5-Chloro-2-nitrobenzylidenebisformamide.—5-Chloro-2-nitrobenzaldehyde (2 g.) (Mettler, *Ber.*, 1905, **38**, 2807) and formamide (10 c.c.) were treated with gaseous hydrogen chloride at 55°. Rapid dissolution occurred, heat was generated, and a solid quickly separated. The mixture was set aside for 2 hours, ethanol added (25 c.c.), and the product collected. Recrystallisation from boiling water gave *5-chloro-2-*

nitrobenzylidenebisformamide as white needles, m. p. 206—207° (75%) (Found: C, 42·34; H, 3·4. $C_9H_8O_4N_2Cl$ requires C, 41·97; H, 3·1%).

6-*Chloroquinazoline*.—(*a*) 6-Aminoquinazoline (0·1 g.; Elderfield *et al.*, *J. Org. Chem.*, 1947, **12**, 405), in hydrochloric acid (2 c.c. of 5N.) was diazotised at 0° with 5% aqueous sodium nitrite, and the solution treated with cuprous chloride (from 0·20 g. of copper sulphate; *Org. Synth.*, Coll. Vol. II, p. 130). After 4 hours at room temperature the mixture was warmed to 80°, cooled, basified, and extracted with ether. Removal of the ether after drying (Na_2SO_4) gave a white solid (0·11 g.), m. p. 143° after crystallisation from ether–ligroin (b. p. 40—60°), identical with the compound described in (*b*).

(*b*) The benzylidenebisformamide (1·5 g.) and zinc dust (4·3 g.) were treated with glacial acetic acid (5·7 g.) and crushed ice (18 g.) during 10 minutes. The flask was shaken for ½ hour, and, after stirring for a further 1½ hours with addition of small amounts of zinc dust (1·5 g. in all), the mixture was filtered, basified with sodium hydroxide, and extracted with ether. Removal of the ether after drying (Na_2SO_4) gave 6-*chloroquinazoline*, which crystallised from ether–ligroin (b. p. 40—60°) or from water as white needles, m. p. 143° (84%) (Found: C, 58·35; H, 3·3. $C_8H_5N_2Cl$ requires C, 58·38; H, 3·1%).

Nitration of 4-*Methylcinnoline*.—4-Methylcinnoline (0·5 g.) in concentrated sulphuric acid (2 c.c.) at 0° was treated with 1 c.c. of a nitrating mixture [from 1·85 c.c. of nitric acid (*d* 1·5) and 8·15 c.c. of concentrated sulphuric acid], added with stirring during 5 minutes. Stirring was continued for ½ hour at 0° and 2 hours at room temperature, the solution poured on ice and basified with ammonia, and the product (0·47 g.) crystallised from methanol, giving 8-*nitro*-4-*methylcinnoline* (0·32 g.; 49%), m. p. 138—139° (decomp.) (Found: C, 57·03; H, 3·6. $C_9H_7O_2N_3$ requires C, 57·12; H, 3·7%). The compound is difficult to free from a brown discoloration, but, when this is achieved, it gives yellow plates from methanol.

8-*Amino*-4-*methylcinnoline*.—The nitro-compound (0·5 g.) in hydrochloric acid (10 c.c. of 6N.) was treated with stannous chloride (2·5 g.) in concentrated hydrochloric acid (2·5 c.c.) at 50° for 10 minutes, and the mixture cooled, basified with concentrated potassium hydroxide solution, and extracted with ether. Removal of the ether after drying (Na_2SO_4) gave a yellow solid (0·38 g.), which formed orange prisms of 8-*amino*-4-*methylcinnoline*, m. p. 126—127°, on crystallisation from ether–ligroin (b. p. 40—60°) (Found: C, 67·64; H, 5·8; N, 26·2. $C_9H_9N_3$ requires C, 67·89; H, 5·7; N, 26·3%).

2-*Nitro*-3-*aminoacetophenone*.—When hydrolysing 2-nitro-3-acetamidoacetophenone to the amine (cf. Leonard and Boyd, *J. Org. Chem.*, 1946, **11**, 409) it proved essential to stop the reaction as soon as the acetamido-compound had dissolved in the acid, otherwise tars were formed and the required amine could not be isolated.

2-(3′-*Chloro*-2′-*aminophenyl*)*propan*-2-*ol*.—3-Chloro-2-aminoacetephenone (3 g.; from the amine described above, by the method of Simpson *et al.*, *J.*, 1945, 646) in ether (50 c.c.) was added during 10 minutes to a stirred Grignard reagent (from 1·71 g. of magnesium, 10·02 g. of methyl iodide, and 100 c.c. of ether) at room temperature. The resulting suspension was stirred and refluxed for ½ hour, decomposed with ice and ammonium chloride, and extracted with ether. Removal of the ether after drying (Na_2SO_4) gave a yellow oil (3·2 g.). No crystalline product could be prepared from this material with acetyl chloride–pyridine, acetic anhydride, or picric acid. With phenyl *iso*cyanate a white solid resulted, but was not examined further.

2-(3′-*Chloro*-2′-*aminophenyl*)*prop*-1-*ene*.—The above-named carbinol (3·1 g.), phosphoric oxide (6·2 g.), and benzene (50 c.c.) were heated under reflux for 3 hours, and the mixture decomposed with ice, basified with ammonia, and extracted with ether. Removal of the solvent after drying (Na_2SO_4) gave a yellow oil (2·7 g.). Treatment for 1 hour at 95° with acetic anhydride converted this into 2-(3′-*chloro*-2′-*acetamidophenyl*)*prop*-1-*ene*, which formed crisp, colourless prisms, m. p. 125—126°, from ether–ligroin (b. p. 40—60°) (Found: C, 62·88; H, 5·9. $C_{11}H_{12}ONCl$ requires C, 62·99; H, 5·8%).

8-*Chloro*-4-*methylcinnoline*.—(*a*) 8-Amino-4-methylcinnoline (0·2 g.) in hydrochloric acid (2 c.c. of 6N.) was diazotised at 0° with 5% aqueous sodium nitrite, and the solution treated dropwise with cuprous chloride solution (from 0·36 g. of copper sulphate). After 4 hours at room temperature the mixture was heated to 80°, cooled, basified, and extracted with ether. Removal of the solvent from the dried (Na_2SO_4) extract, and crystallisation of the residue (0·05 g.) from ligroin (b. p. 60—80°) gave a product, m. p. 126—127°, identical with that described in (*b*).

(*b*) The olefin described above (from 3·2 g. of carbinol) was dissolved in hydrochloric acid (16 c.c. of 2N- and 5 c.c. of concentrated acid) and diazotised at 0° with sodium nitrite (1·3 g. in 5 c.c. of water). When kept at room temperature, the solution became red and finally green. After 8 days, some tar had appeared, and addition of sodium hydroxide solution precipitated first more tar and then a grey solid. The latter was collected and, by extracting the tar with warm ether, a little more was obtained. The combined crops (1·44 g.) were extracted with ligroin (b. p. 60—80°) in a Soxhlet apparatus, a dark blue solid (0·37 g.; m. p. indefinite) remaining undissolved, and concentration of the extract gave a yellow solid (0·9 g.). Several crystallisations of this last from ligroin (b. p. 60—80°) gave yellow needles of 8-*chloro*-4-*methylcinnoline*, m. p. 126—127° (Found: C, 60·23; H, 4·1. $C_9H_7N_2Cl$ requires C, 60·50; H, 3·9%). The *picrate* formed stout green needles from ethanol; several crystallisations from ethanol served to decrease the intensity of the colour without removing it completely; the pure compound had m. p. 179—180° (decomp.) (Found: C, 44·86; H, 2·3. $C_{15}H_{10}O_7N_5Cl$ requires C, 44·20; H, 2·5%). The formation of strongly coloured impurities has been observed in other examples of this reaction (Atkinson and Simpson, *J.*, 1947, 808; Jacobs *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 1310), and it is possible that the reported colours of 4-methylcinnoline picrates are due to impurities, since 8-chloro-4-methylcinnoline picrate prepared from material obtained by nitration appeared to be yellow although available quantities were too small fully to establish this point.

o-*Formamidoacetophenone*.—*o*-Aminoacetophenone (6 g.) was boiled under reflux for 10 minutes with anhydrous formic acid (8 g.), the solution poured into water, and the precipitate collected; the product (4·73 g.) had m. p. 77—78° (Camps, *Ber.*, 1901, **34**, 2703, gives m. p. 79°).

4-*Methylquinazoline*.—The formamido-compound (2 g.), aqueous ammonia (4 c.c.; *d* 0·880), and alcohol (10 c.c.) were heated in a sealed tube for 4 hours at 140°, and the solution concentrated, giving a pale brown oil which did not crystallise. Distillation gave 4-methylquinazoline (1·32 g., b. p.

126—128/12 mm.) which crystallised in the ice-chest. It was characterised as the picrate, m. p. 181·5—182° (Bogert and Nabenhauer, *J. Amer. Chem. Soc.*, 1924, **46**, 1932, give m. p. 183·5°) (Found: C, 48·5; H, 3·3. Calc. for $C_{15}H_{11}O_7N_5$: C, 48·3; H, 3·0%).

Nitration of 4-Hydroxyquinoline.—(*a*) Nitration under the conditions given by Mosher and his co-workers (*J. Amer. Chem. Soc.*, 1947, **69**, 303) proceeded as they describe. The derived chloro-nitroquinoline, m. p. 144—145°, was identical with 4-chloro-6-nitroquinoline, m. p. 144—145° (Riegel *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 1264), and under the conditions described below gave 6-nitro-4-phenoxyquinoline, identical with the authentic compound.

(*b*) 4-Hydroxyquinoline (1 g.) in nitric acid (10 c.c.; *d* 1·42) was heated at 95° for 1 hour. Dilution of the solution with ice gave a yellow solid, m. p. (320) 326—328° (0·82 g.), which was boiled under reflux for 2 hours with phosphorus oxychloride (8 c.c.); the solution was decomposed with ice, neutralised with sodium acetate, and extracted with ether. Removal of the solvent after drying (Na_2SO_4) provided a yellow solid (0·79 g.), m. p. (95) 101—104°, which on warming in methanol (20 c.c.) rapidly precipitated a white solid (0·54 g.), m. p. >310°. Rechlorination with phosphorus oxychloride, and crystallisation of the product from ether–ligroin (b. p. 40—60°) gave 4-chloro-3-nitroquinoline, m. p. 119—120°, identical with an authentic specimen (Bachman *et al.*, *J. Amer. Chem. Soc.*, 1947, **68**, 365). This was readily converted into 3-nitro-4-phenoxyquinoline as described below.

3-Nitro-4-phenoxyquinoline.—4-Chloro-3-nitroquinoline (0·65 g.) and phenol (3 g.) were heated at 180° for 4 hours (cf. Backeberg and Marais, *J.*, 1942, 381), and the solution poured into sodium hydroxide solution (100 c.c. of 2N.) and extracted with ether. Removal of the ether after drying (Na_2CO_3) gave a pale yellow solid (0·5 g.). *3-Nitro-4-phenoxyquinoline* separated from ether–ligroin (b. p. 40—60°) in pale yellow needles, m. p. 108—109° (Found: C, 64·11; H, 4·31. $C_{15}H_{10}O_3N_2,H_2O$ requires C, 63·41; H, 4·26%).

6-Nitro-4-phenoxyquinoline.—In the same way, 4-chloro-6-nitroquinoline (0·3 g.) gave *6-nitro-4-phenoxyquinoline* (0·28 g.), forming colourless plates from ether–ligroin (b. p. 40—60°), m. p. 117—118° (Found: C, 66·6; H, 4·0. $C_{15}H_{10}O_3N_2$ requires C, 67·6; H, 3·8%).

Nitration of 1-Methyl-4-cinnolone.—The compound (0·5 g.) in concentrated sulphuric acid (2 c.c.) was treated during 15 minutes at 0° with 1 c.c. of nitration mixture [from 1·6 c.c. of nitric acid (*d* 1·5) and 8·4 c.c. of concentrated sulphuric acid] with stirring, and the solution stirred for a further 15 minutes at 0° and 1½ hours at room temperature, and poured on ice. Recrystallisation of the granular precipitate (0·47 g.) from methanol gave glistening, yellow plates of 8-nitro-1-methyl-4-cinnolone, m. p. 238—239°, identical with a specimen from 8-nitro-4-hydroxycinnoline (Schofield and Theobald, unpublished).

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WASHINGTON SINGER LABORATORIES,
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291. *The Colorimetric Measurement of* pH, *and the Dissociation Constants of the Malonates of Some Bivalent Metals.*

By D. I. STOCK and C. W. DAVIES.

A colorimetric method is described of measuring the pH of an aqueous solution with an accuracy of 0·01 pH unit. It is emphasised that the apparent " pH " measured with sulphonephthalein indicators is not the same quantity as that obtained electrometrically: the relationship between the quantities measured in these two ways is illustrated.

The dissociation constants of the malonates of a number of bivalent metals, in aqueous solution, are reported and discussed.

THE development of the glass electrode, and of pH meters incorporating it, has undoubtedly made the electrometric method the most general method of pH measurement, since it can be applied in the presence of colloids, and in coloured and highly dilute solutions where indicators are inapplicable or inconvenient. Apart from these special cases, however, indicators offer certain advantages, and it seems to us a mistake to regard the electrometric method as the standard, and as always the most accurate, method. A simple comparator being used, indicators readily give a reproducibility of 0·01 pH unit, and although the potentiometric technique can be refined to yield results which are reproducible to a third decimal place, this advantage is offset by the difficulties in interpreting the results, owing to uncertainties in the magnitude of liquid-junction potentials and activity corrections.

Bromophenol-blue was the indicator used in the present work, and our object was to establish its accuracy in measuring true hydrogen-ion concentrations, and then to apply it to the study of ion association in malonate solutions. The usual buffer standards could not be employed, because, although their pH values are known with great accuracy, the interpretation of these

is attended by the uncertainties already mentioned. Instead, we used as standards partly neutralised solutions of weak acids, the hydrogen-ion concentrations of which could be calculated with a high degree of accuracy from known dissociation constants.

The Measurement of Hydrogen-ion Concentration with Bromophenol-blue.—The main colour change of the sulphonephthalein indicators is known to be associated with the ionisation of a phenolic hydrogen in the singly-charged yellow carboxylate ion (cf. Kolthoff, *J. Physical Chem.*, 1931, **35**, 1433). For bromophenol-blue, therefore, the indicator equilibrium will be controlled by the equation $f_{H^+} \cdot f_{B''}[H^+][B''] / f_{HB'}[HB'] = K_I$, and the colour of the indicator will be governed by the ratio [B″]/[HB′]. Consequently, when we compare two solutions in which the concentration and colour of the indicator are identical, the quantity $f_{H^+} \cdot f_{B''}[H^+] / f_{HB'}$ is the same in each. At sufficiently low ionic strengths, the ion-activity coefficients may be evaluated by means of the Debye–Hückel limiting equation, or by an empirical extension of this; for instance, at ionic strengths not exceeding 0·05, there are good grounds for believing that departures (at 25°) from the equation $\log f_i = -0{\cdot}5 z_i^2 \{\sqrt{I}/(1 + \sqrt{I}) - 0{\cdot}2I\}$ (Davies, *J.*, 1938, 2093) will be well within the accuracy of 2% at which we were aiming. The activity coefficient of the univalent ions being taken as equal, and that of the bivalent ion B″, *i.e.*, $f_{B''}$, being written as f_2, equality of colour in two solutions will indicate identity of the quantity $pf_2[H^+] = -\log f_2[H^+]$, or, after introduction of the equation above, of the quantity

$$pf_2[H^+] = -\log [H^+] + 2\{\sqrt{I}/(1 + \sqrt{I}) - 0{\cdot}2\, I\}.$$

We have confirmed this conclusion by preparing two buffer solutions of known ionic composition and widely differing ionic strengths, which gave identical colours with the standard concentration of indicator. The first solution was 0·004931M with respect to sodium hydrogen malonate and 0·03304M with respect to potassium chloride. By using German and Vogel's values for the dissociation constants of malonic acid ($K_1 = 1{\cdot}4 \times 10^{-3}$; $K_2 = 2{\cdot}2 \times 10^{-6}$; *J. Amer. Chem. Soc.*, 1936, **58**, 1546), the hydrogen-ion concentration can be calculated to be $7{\cdot}86 \times 10^{-5}$ mol./l., the ionic strength is 0·03831, and $pf_2[H^+] = 4{\cdot}42$. The second solution was 0·005850M with respect to sodium benzoate and 0·004012M with respect to benzoic acid, and for this, $[H^+] = 5{\cdot}09 \times 10^{-5}$, $I = 0{\cdot}005850$, and $pf_2[H^+] = 4{\cdot}43$. It will be seen that the agreement between the calculated $pf_2[H^+]$ values is satisfactory, although the hydrogen-ion concentration of the first solution is more than 50% greater than that of the second. If we use what is perhaps the most usual definition of pH, *viz.*, $pH = -\log f_1[H^+]$, where f_1 is a mean value for univalent ions, then the pH of the first solution is 4·18 and that of the second is 4·33, a difference of 0·15 pH unit. This illustrates the errors that may be involved in basing colorimetric measurements on electrometric standards. It may be noted that similar, though smaller, discrepancies arise when monobasic acids, such as phenolphthalein are used as indicators. From the equation $f_{H^+} \cdot f_{P'}[H^+][P'] / [HP] = K_I$, it will be clear that in this case the colour of the indicator is a measure of the quantity $f_1^2[H^+]$. Though the correct interpretation of colorimetric pH measurements has been established before (see, for instance, Guggenheim and Schindler, *J. Physical Chem.*, 1934, **38**, 543), it is still frequently found that equality of indicator colour is either taken to imply pH equality (whereas with no type of indicator does the quantity studied correspond to the electrometrically measured pH), or else that the error involved in ignoring activity corrections is negligible. As the example quoted demonstrates, this error may well be large, and now that some reliance can be placed on the calculation of activity coefficients in dilute solutions it seems unfortunate that these differences in scale should often be ignored, or disguised as part of a " salt error."

Ion Association in Malonate Solutions.—Malonic acid, sodium hydroxide, and metal chloride solutions were mixed to give the final concentrations: NaHMal 0·004931M, MCl_2 0·01233M, the standard amount of indicator being added. The colour of the solution was then matched by a standard benzoate or mandelate buffer, thus determining the value of $pf_2[H^+]$. The results obtained are shown in Table I, col. 1 showing the cation added to the malonate buffer, and col. 2 giving the $pf_2[H^+]$ value. The effect of adding the bivalent chloride is invariably to increase the hydrogen-ion concentration of the " buffer "—by more than 20-fold in the case of copper—a result which is primarily due to the reaction $M^{\cdot\cdot} + HMal' = MMal + H^+$. The amount of undissociated metal malonate is calculated as follows. The solution contains, besides water and indicator, the following species: Na^+, Cl', $M^{\cdot\cdot}$, MMal, Mal″, HMal′, H_2Mal, H^+, OH', and possibly $MHMal^+$ (sodium malonate and the metal chlorides are known to be highly dissociated). Of the ions specified, the concentrations of Na^+ and Cl' are known, that of OH' is negligible in these acid solutions, and that of $MHMal^+$, though unknown, is unlikely to be large enough to affect the relative concentrations of the other species. We are left with six unknowns, which can be

evaluated by successive approximations from (i) the $pf_2[H^+]$ value, (ii) the equation for electroneutrality, (iii) the known total concentration of malonate radical, (iv) the known total concentration of bivalent metal, and (v) and (vi) the K_1 and K_2 values of malonic acid. The results of the calculations are given in cols. 3—8 of Table I, in millimols./l., and col. 9 shows the ionic strength. Col. 10 gives the dissociation constant of the metal malonate, calculated from the equation $K = f_2^2[M^{\cdot\cdot}][Mal'']/[MMal]$, and col. 11 gives values previously derived from conductivity measurements (Riley and Fisher, *J.*, 1929, 2006; Ives and Riley, *J.*, 1931, 1998; Money and Davies, *Trans. Faraday Soc.*, 1932, **28**, 609).

TABLE I.

Cation.	pf_2 [H·].	[H·].	[HMal'].	[Mal''].	[H_2Mal].	[MMal].	[M··].	*I*.	$K \cdot 10^4$.	$K \cdot 10^4$ (cond.).
Ba··.....	4·39	0·086	4·47	0·241	0·189	0·034	12·3	0·0421	196	—
Ca··.....	4·28	0·109	4·36	0·184	0·235	0·157	12·2	0·0417	32	—
Mg·· ...	4·19	0·137	4·23	0·143	0·284	0·277	12·1	0·0413	14	16
Cd··.....	4·04	0·191	3·99	0·0962	0·376	0·470	11·9	0·0408	5·6	5·1
Mn·· ...	$4·02_5$	0·197	3·96	0·0923	0·386	0·493	11·8	0·0407	5·1	—
Co·· ...	3·84	0·299	3·57	0·0546	0·530	0·773	11·6	0·0399	1·9	—
Ni·· ...	3·72	0·395	3·26	0·0375	0·640	0·997	11·3	0·0394	0·99	0·73
Cu··.....	$3·07_5$	1·69	1·20	0·0031	1·021	2·71	9·62	0·0348	0·028	0·025

The results obtained by the two methods are in satisfactory agreement, and show a strong variation in the dissociation constants of the malonates, from the barium salt where ion association is less important than in the sulphates so far studied (Davies, *Trans. Faraday Soc.*, 1927, **23**, 351; Owen and Gurry, *J. Amer. Chem. Soc.*, 1938, **60**, 3074), to the weak copper salt which will be only 1% dissociated at 0·025M.

As a first step in interpreting these results we may consider them in the light of Bjerrum's theory of ion association (*Kgl. Danske Vidensk. Selskab.*, 1926, **7**, No. 9). On the assumption that ion association is wholly due to the Coulomb attraction between oppositely charged ions, this theory gives a relation between the dissociation constant and the sum (a) of the radii of the associating ions, which must be assumed spherical and with the charge located at the centre. Table II shows the a values derived in this way from our dissociation constants, and also the cationic radii (r) given by Pauling ("Nature of the Chemical Bond," Chap. X). There is a rough parallelism between the two, but clearly, other factors are also operative, such as variations, not directly related to radius, in the free energy of hydration of the ions. The large mean ionic diameter found for barium malonate may be explained by the fact that the assumption of spherical symmetry and a central charge is far from the truth for the malonate ion; the a values for some of the other salts then become, by comparison, impossibly small, and indicate that for these cations also the assumptions are inadequate.

TABLE II.

Cation.	Ba··.	Ca··.	Mg··.	Cd··.	Mn··.	Co··.	Ni··.	Cu··.
a (A.)	10·0	3·74	2·9	2·5	2·4	2·2	2·0	1·5
r (A.)	1·35	0·99	0·65	0·97	0·80	0·72	0·70	—

It is also noteworthy that the order of stability of the undissociated complexes between the bivalent cations and the malonate radical found in this work, as well as in an earlier study of metal exalates (*Trans. Faraday Soc.*, 1932, **28**, 613), is substantially the same (with one exception) as that discussed by Mellor and Maley (*Nature*, 1948, **161**, 436) and Irving and Williams (*ibid.*, 1948, **162**, 746): the complexes referred to by these workers were chelate in character, and it is stated that for such complexes of bivalent metal ions the stability follows the order given, irrespective of the nature of the ligands involved. That the oxalates and malonates also conform to this order may perhaps be a further indication that the linkage here is also largely covalent, and not merely due to (Coulomb) ion association.

EXPERIMENTAL.

Colour comparisons were made visually in a north light, using a Hellige comparator. The acids were pure specimens, recrystallised three times from conductivity water. The salts were of "AnalaR" grade, used without further purification. Sodium hydroxide was prepared from clean pieces of sodium, which were allowed to react in a platinum dish standing over carbon dioxide-free conductivity water in a vacuum desiccator; the resulting solution was transferred, diluted, and stored with the necessary precautions in a nickel bottle, and was standardised, through hydrochloric acid, against "AnalaR" sodium carbonate. Bromophenol-blue was used as received from B.D.H. Ltd. at a concentration of 1·38 ml. of 0·04% solution in 100 ml. of solution.

The colour standards consisted of 0·01N-mandelic or benzoic acid, partly neutralised by sodium hydroxide or sodium carbonate; that carbonic acid does not interfere at the pH values studied was proved both experimentally and by calculation. Mandelic and benzoic acids were chosen because their dissociation constants are known with high accuracy from conductivity investigations which have also shown ion association to be negligible in dilute solutions of the sodium salts. In making a colour comparison, the standard amount of indicator was added to the solution under test, and 25 ml. of a 0·02N-solution of mandelic or benzoic acid, plus indicator, were then titrated with alkali, with simultaneous additions of conductivity water, to give 50 ml. of a solution of identical colour. This was repeated three times, and the results always agreed to within 0·01 pH unit, one drop of alkali producing a perceptible colour change. The $pf_3[H^+]$ value of the solutions was then interpolated from a graph connecting $pf_3[H^+]$ with the amount of alkali added. This graph was constructed from the activity equation already quoted and the dissociation constant of the acid (mandelic, $K = 3{\cdot}88 \times 10^{-4}$, Banks and Davies, *J.*, 1938, 73; benzoic, $K = 6{\cdot}320 \times 10^{-5}$, Brockman and Kilpatrick, *J. Amer. Chem. Soc.*, 1934, **56**, 1485).

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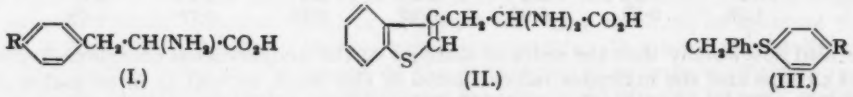
292. *Bacteriostasis in the Amino-acid Series. Part II. Further Studies with Alanine Derivatives.*

By D. F. ELLIOTT and SIR CHARLES HARINGTON.

The search for antibacterial activity in the amino-acid group has been continued. The synthesis of several more alanine derivatives with widely different structures is described. One only of these amino-acids, β-3-thianaphthenylalanine, exerted a significant bacteriostatic action, and this was confined to *Strep. hæmolyticus*.

IN continuation and extension of the work described in Part I (*J.*, 1948, 85) the present paper describes the synthesis of derivatives of β-phenylalanine substituted in the *p*-position with the following groups: guanidino, guanidinomethyl, *p*-aminophenylsulphonyl, and mercapto. The first two were selected in further development of the previous investigation of the effect of basic substituents in conferring bacteriostatic action on alanine derivatives; attempted extension of this line of work to the synthesis of *p*-amidino- and *p*-2′-diethylaminoethylamino-derivatives of β-phenylalanine was abandoned owing to preparative difficulties. The amino-phenylsulphonyl derivative seemed worth studying in view of the known tuberculostatic action of certain diaryl sulphones carrying basic substituents.

The *p*-mercaptophenylalanine (I; R = SH) was considered to be a possible tyrosine antagonist which might therefore inhibit the growth of micro-organisms with obligatory requirements for aromatic amino-acids. With a similar thought in mind, a possible inhibitor of tryptophan metabolism, namely β-3-*thianaphthenylalanine* (II) has been prepared and its synthesis is here described. Since our work was completed, a different synthesis of this amino-acid has been published by Avakian, Moss, and Martin (*J. Amer. Chem. Soc.*, 1948, **70**, 3075).



(I.) (II.) (III.)

β-p-*Guanidinophenylalanine* [I; R = NH·C(NH$_2$):NH] was prepared by the action of cyanamide on *ethyl acetamido-4-aminobenzylmalonate*, followed by acid hydrolysis of the product. The homologous guanidinomethyl derivative [I; R = ·CH$_2$·NH·C(NH$_2$):NH] was obtained by acid hydrolysis of the product of condensation of ethyl acetamido-4-aminomethylbenzylmalonate (Part I, *loc. cit.*) with *S*-methyl*iso*thiourea; difficulties were encountered in effecting this condensation until it was found that the reaction proceeded smoothly in phenol solution at 115°.

In the synthesis of β-p-(p′-*aminophenylsulphonyl*)*phenylalanine* (I; R=*p*-NH$_2$·C$_6$H$_4$·SO$_2$·), *p*-nitrophenyl *p*-tolyl sulphide (Law and Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 3625) was oxidised with hydrogen peroxide to give the corresponding *sulphone*. This sulphone was brominated in the ω-position, and the product was condensed with ethyl acetamidomalonate; the resulting *ester*, on hydrolysis with hydrobromic and acetic acid, was smoothly converted into the *amino-acid* (I; R = *p*-NO$_2$·C$_6$H$_4$·SO$_2$). Catalytic reduction then yielded the desired aminophenylsulphonylphenylalanine.

β-*p*-Mercaptophenylalanine has already been prepared by a somewhat cumbersome method

(Johnson and Brautlecht, *J. Biol. Chem.*, 1912, **12**, 175). The procedure now described offers advantages of ease of manipulation. The starting point of the synthesis was 4 : 4'-dicarboxydiphenyl disulphide; by reduction with sodium in liquid ammonia, followed by treatment with benzyl chloride, this compound was converted in one operation into p-*benzylthiobenzoic acid* (III; R = CO_2H). The derived *ethyl* ester was converted successively into the *hydrazide* and *toluene*-p-*sulphonhydrazide*; the sulphonhydrazide, under the conditions prescribed by McFadyen and Stevens (*J.*, 1936, 584), yielded p-*benzylthiobenzaldehyde* (III; R = CHO). The aldehyde was condensed with hippuric acid, and the resulting *oxazolone* hydrolysed to *α-benzamido*-p-*benzylthiocinnamic acid* [III; R = $CH{:}C(NHBz){\cdot}CO_2H$]. On reductive hydrolysis, by boiling with hydriodic acid and phosphorus, the latter acid yielded the desired β-*p*-mercaptophenylalanine (I; R = SH). In the process of removal of hydriodic acid some oxidation to the disulphide invariably occurred; it was therefore convenient to complete this oxidation by means of iodine and to isolate the disulphide, from which the thiol could readily be obtained by reduction. The mercapto-amino-acid was obtained as the *hydrochloride dihydrate*, which was not described by Johnson and Brautlecht (*loc. cit.*).

For the synthesis of β-3-thianaphthenylalanine the starting point was thianaphthen-3-aldehyde. This aldehyde had been prepared in poor yield by Komppa and Weckmann (*J. pr. Chem.*, 1933, **138**, 109) by the action of ethyl orthoformate or ethoxymethyleneaniline on 3-thianaphthenylmagnesium bromide. We obtained moderate yields of the aldehyde both by the Rosenmund reduction of thianaphthen-3-carboxyl chloride and by the McFadyen–Stevens reduction of the corresponding *thianaphthen-3-carboxy*-p-*toluenesulphonhydrazide*. The aldehyde was condensed with hippuric acid in the usual way and the *oxazolone* reduced directly to *benzoyl*-β-3-*thianaphthenylalanine* by the method of Lamb and Robson (*Biochem. J.*, 1931, **25**, 1234). Hydrolysis with a mixture of hydrochloric and acetic acids gave the amino-acid (II).

Biological Results.—None of the above-mentioned amino-acids inhibited the growth of *S. aureus* or *E. coli* in broth up to the limit of their solubility. Against *Strept. hæmolyticus* in broth, (II) inhibited growth at a dilution of 1 in 20,000. The other amino-acids were very weakly active. We are indebted to Dr. A. T. Fuller for these results. Dr. P. D'Arcy Hart has also tested (I; R = *p*-$NH_2{\cdot}C_6H_4{\cdot}SO_2$) against *M. tuberculosis*; the compound has slight tuberculostatic action *in vitro*, but is less effective than *pp'*-diaminodiphenyl sulphone.

Experimental.

(M. p.s are uncorrected.)

Ethyl Acetamido-4-nitrobenzylmalonate.—To a solution of sodium (1·15 g.) in dry alcohol (25 c.c.) was added ethyl acetamidomalonate (10·9 g.), and, when the ester had dissolved, a hot solution of 4-nitrobenzyl bromide (11·3 g.) in alcohol (25 c.c.) was added in one portion. When the vigorous reaction had subsided, the mixture was boiled under reflux for 15 minutes and then diluted with water. The *ester* was filtered off, washed with water, and crystallised from alcohol. Yield, quantitative; m. p. 190° (Found: N, 7·95. $C_{16}H_{20}O_7N_2$ requires N, 8·0%).

Ethyl Acetamido-4-aminobenzylmalonate.—The above nitro-compound (3·5 g.) was suspended in alcohol (100 c.c.), Raney nickel (1 g.) was added, and the mixture hydrogenated at room temperature and pressure. Complete hydrogenation of the nitro-group required 6 hours owing to the sparing solubility of the nitro-compound. The solution was filtered and evaporated to dryness under reduced pressure, and the residue crystallised from benzene. It formed colourless prisms, m. p. 132°. Yield, 2·8 g. (87%) (Found: N, 8·8. $C_{16}H_{22}O_5N_2$ requires N, 8·7%).

Ethyl Acetamido-4-guanidinobenzylmalonate Picrate.—The amine (4·8 g.) and cyanamide (1·5 g.) were heated under reflux in alcohol (60 c.c.) containing one equivalent of hydrogen chloride for 8 hours. The solution was then evaporated to dryness under reduced pressure, and the residual oil lixiviated with cold water (*ca.* 30 c.c.) and filtered. A large excess of solid ammonium nitrate was added to the filtrate. Next day the crude nitrate was collected and dissolved in hot water (*ca.* 250 c.c.), and a hot aqueous solution of ammonium picrate added in excess. When cold, the *picrate* was filtered off and crystallised several times from alcohol. It formed rectangular plates, m. p. 190—191° (4·8 g.) (Found: N, 16·2. $C_{23}H_{27}O_{12}N_7$ requires N, 16·5%).

β-p-Guanidinophenylalanine Monohydrochloride.—The pure picrate described above (3 g.) was boiled under reflux for 4 hours with 3N-hydrochloric acid (20 c.c.). The picric acid was then removed by several extractions with warm benzene, and the aqueous layer evaporated to dryness under reduced pressure. The residual syrup was dissolved in 2 c.c. of hot water, and the solution neutralised to pH 4 with concentrated aqueous ammonia. The crude *monohydrochloride* (1·1 g.) which separated on cooling could not be obtained pure by recrystallisation. 1·3 G. were dissolved in hot water (15 c.c.), and a solution of ammonium picrate (1·5 g.) in hot water (15 c.c.) was added. When the solution cooled, a mixture of orange prisms and pale-yellow needles separated. The whole was then carefully reheated, with stirring, until the yellow needles had redissolved. The liquid was decanted, and the orange prisms remaining were recrystallised several times from hot water. The pure *monopicrate* had m. p. 226—228° (decomp.) (Found: C, 42·85; H, 3·7; N, 21·8. $C_{16}H_{17}O_9N_7$ requires C, 42·6; H, 3·8; N, 21·7%). The pure monohydrochloride was obtained from the monopicrate (1·45 g.) by adding it to 3N-hydrochloric acid (4 c.c.), extracting several times with warm benzene, evaporating it to dryness under reduced

pressure, redissolving the syrup in hot water (1 c.c.), and adding concentrated aqueous ammonia to pH 4, followed by alcohol (3 c.c.). The monohydrochloride separated in hexahedral crystals, m. p. 240—242° (decomp.) (Found : N, 21·0. $C_{10}H_{15}O_3N_4Cl$ requires N, 21·7%). The Sakaguchi and the ninhydrin reaction were positive.

Ethyl Acetamido-4-guanidinomethylbenzylmalonate Picrate.—Ethyl acetamido-4-aminomethylbenzylmalonate hydrochloride (Part I, *loc. cit.*; 14·8 g.) was added to one equivalent of ice-cold N-sodium hydroxide, the free base extracted with chloroform, and the chloroform extract dried and evaporated under reduced pressure without heating. The residue was dissolved in warm phenol (40 g.), *S*-methyl-*iso*thiourea sulphate (5·6 g.) added, and the mixture heated to 115°. The sulphate slowly dissolved with evolution of methylthiol. The mixture was occasionally shaken. After the mixture had been maintained for 2½ hours at 115—120°, the homogeneous solution was cooled, water (100 c.c.) added, and the phenol shaken out with ether. The lower layer was warmed under reduced pressure for a short while to remove ether, excess ammonium nitrate added, and the solution acidified with a few drops of dilute nitric acid. The crude nitrate which separated was filtered off and dissolved in hot water (200 c.c.), and a hot concentrated solution of ammonium picrate added until precipitation was complete. The crystalline solid was collected, washed by stirring with several small portions of hot water and filteng each time, and crystallised three times from alcohol. The pure picrate had m. p. 169—171°. Yield, 9·2 g. (38%) (Found : N, 15·9; OEt, 14·8. $C_{24}H_{29}O_{12}N_7$ requires N, 16·15; OEt, 14·8%).

β-p-*Guanidinomethylphenylalanine Monohydrochloride.*—The pure picrate described above (3 g.) was heated under reflux for 4 hours with 3N-hydrochloric acid (20 c.c.), and the solution extracted several times with warm benzene to remove picric acid. The aqueous layer was evaporated to dryness under reduced pressure, the residue dissolved in 5 c.c. of hot water, and the solution adjusted to pH 5 with concentrated ammonia solution. After 24 hours at 0° the *monohydrochloride* was filtered off and recrystallised from hot water. It formed rectangular plates, m. p. 290—292° (decomp.) [yield, 1·2 g. (88%)] (Found : C, 48·4; H, 6·3; N, 20·45. $C_{11}H_{17}O_2N_4Cl$ requires C, 48·4; H, 6·3; N, 20·5%). The Sakaguchi and the ninhydrin test were positive.

p-*Nitrophenyl* p-*Tolyl Sulphone.*—*p*-Nitrophenyl *p*-tolyl sulphide (Law and Johnson, *loc. cit.*; 19 g.) was dissolved with gentle warming in acetic acid (150 c.c.); the solution was treated with hydrogen peroxide (36 c.c. of 30%) which caused some precipitation; the mixture was cautiously warmed until reaction set in, and external heat was then withdrawn; after subsidence of the vigorous effervescence the solution was boiled for 2 hours and allowed to cool. The *sulphone* which separated (19·3 g.) was almost pure. After recrystallisation from acetic acid it formed stout pale yellow prisms, m. p. 176° (Found : S, 11·1. $C_{13}H_{11}O_4NS$ requires S, 11·5%).

p-*Nitrophenyl ω-Bromo*-p-*tolyl Sulphone.*—The preparation of this compound offered difficulty. With 1 mole of bromine a product containing only about 75% of the theoretical amount of bromine was obtained; on the other hand, if addition of bromine were carried further, some dibromination occurred. Best results were achieved by carrying out the reaction in two stages as follows. The sulphone (14 g.) was melted and mechanically stirred in an oil-bath at about 190°; bromine (8 g.) was added slowly, and the melt allowed to cool. Recrystallisation from acetic acid gave 14 g. of a product having m. p. 170° and containing 16% of bromine (theory, 22·5%). This product (12·85 g.) was again melted and treated with bromine in slight excess over the theoretical amount for completion of the reaction, the addition being interrupted when a fresh drop of bromine ceased to cause a brisk evolution of hydrogen bromide. Crystallisation of the reaction product first from acetic acid and then from methyl ethyl ketone yielded 8·7 g. of *bromo*-derivative having m. p. 174—176°. Even this sample was not quite pure, as shown by the analysis (Found : C, 44·7; H, 3·1; Br, 20·1. $C_{13}H_{10}O_4NSBr$ requires C, 43·8; H, 2·8; Br, 22·5%); it was, however, satisfactory for further reaction.

Ethyl Acetamido-p-(p′-*nitrophenylsulphonyl*)*benzylmalonate.*—Ethyl acetamidomalonate (4 g.) was dissolved in a solution of sodium (0·42 g.) in anhydrous alcohol (20 c.c.); the bromo-sulphone (6·5 g.) was dissolved in anhydrous dioxan (20 c.c.), and the solutions were mixed; after refluxing for 3½ hours reaction was complete; the mixture was cooled, diluted with water, and the *ester* collected and crystallised from alcohol. It formed colourless narrow prisms, m. p. 196° [6·63 g. (74%)] (Found : C, 53·3; H, 4·9; N, 6·0. $C_{22}H_{24}O_9N_2S$ requires C, 53·7; H, 4·9; N, 5·7%).

β-p-(p′-*Nitrophenylsulphonyl*)*phenylalanine.*—The preceding ester (6 g.) was dissolved in acetic acid (30 c.c.); hydrobromic acid (30 c.c., *d* 1·7) was added, and the mixture heated under reflux for 4 hours. On cooling, the hydrobromide of the amino-acid separated; it was collected and dried. Yield, 5 g. (95%). The free *amino-acid* was obtained by dissolving the hydrobromide in hot water with addition of hydrobromic acid, and neutralising at the boiling point with aqueous ammonia. It separated in bunches of colourless stumpy needles, m. p. 236—238° (decomp.) (Found : N, 7·6; S, 9·5. $C_{15}H_{14}O_6N_2S$ requires N, 8·0; S, 9·6%).

β-p-(p′-*Aminophenylsulphonyl*)*phenylalanine.*—The hydrobromide of the nitrophenylsulphonylphenylalanine (5 g.) was suspended in 50% alcohol (200 c.c.) to which was added hydrobromic acid (1·35 c.c., *d* 1·49; 1 equivalent). The mixture was hydrogenated at atmospheric pressure in presence of platinum oxide (150 mg.). The uptake of hydrogen became very slow after 3 hours, the reaction being still incomplete. Hydrobromic acid (5 c.c., *d* 1·49) and platinum oxide (100 mg.) were added, and hydrogenation resumed; the uptake finally (about 5 hours in all) ceased at 820 c.c. (theory at 23°/762 mm. is 850 c.c.). The solution was filtered and evaporated to dryness under reduced pressure. The residue was taken up in water, freed from a little black amorphous material by filtration, and neutralised to pH 7—8 at the boiling point by addition of aqueous ammonia. After keeping overnight at 0° the *amino-acid* was collected, washed with water, and dried. Yield, 3·1 g. (83%) of practically pure material. For analysis it was recrystallised by dissolution in water with the aid of ammonia and neutralisation to litmus at the boiling point with acetic acid. It formed colourless plates, m. p. 267—269° (decomp.), and was much more soluble in water than the corresponding nitro-compound (Found : C, 56·2; H, 5·3; N, 8·6; S, 9·8. $C_{15}H_{16}O_4N_2S$ requires C, 56·3; H, 5·0; N, 8·8; S, 10·0%).

p-*Benzylthiobenzoic Acid.*—*pp*′-Dicarboxydiphenyl disulphide was prepared from *p*-aminobenzoic acid according to method described in *Org. Synth.*, Coll. Vol. II, p. 580 for the *oo*′-isomer and was used

in the crude state, containing some salt. An amount of the crude material containing 15 g. of the disulphide was dissolved in liquid ammonia (300 c.c.), and sodium was added in small portions to the well-stirred solution until present in excess. Somewhat more than six atoms were needed. The blue colour caused by excess of sodium was discharged by addition of the minimum amount of ammonium chloride, and benzyl chloride (12·5 c.c.; 50% excess) was stirred in. The ammonia was allowed to evaporate, the residue dissolved in boiling water, and the solution filtered. After the mixture had been kept overnight at 0°, the sodium salt which had separated was collected, dissolved in boiling water, and acidified while hot with hydrochloric acid. The precipitated *acid* [16 g. (66%)] was collected after cooling and dried. It crystallised from acetic acid in colourless prisms, m. p. 189—190° (Found: S, 13·2. $C_{14}H_{12}O_2S$ requires S, 13·1%). The *ethyl* ester was prepared by boiling a solution of the acid (6·62 g.) in alcohol (70 c.c.) containing sulphuric acid (7 c.c.) for 4 hours, and pouring the hot solution into water (500 c.c.) containing sodium bicarbonate (22 g.); the ester was isolated by extraction with ether. Yield, 7 g. (95%). It formed colourless prisms from alcohol, m. p. 60° (Found: S, 12·0. $C_{16}H_{16}O_2S$ requires S, 11·8%).

p-*Benzylthiobenzhydrazide*.—The preceding ethyl ester (5·44 g.) was dissolved in alcohol (10 c.c.) containing hydrazine hydrate (1·5 c.c. of 100%) and the solution heated on the steam-bath in a pressure-bottle for 17 hours; the contents were poured whilst hot into a beaker, and the *hydrazide* which separated on cooling was collected (yield, 4·17 g.; 80%). Recrystallised from alcohol it formed colourless plates, m. p. 166° (Found: N, 11·2; S, 12·7. $C_{14}H_{14}ON_2S$ requires N, 10·9; S, 12·4%). The *toluene*-p-*sulphonyl* derivative was prepared by adding toluene-*p*-sulphonyl chloride (9·7 g.; 10% excess) in small portions to a solution of the hydrazide (11·8 g.) in dry pyridine (120 c.c.) with just enough warming to maintain a clear solution; after heating for 10 minutes on the steam-bath the mixture was poured into ice-water containing excess of hydrochloric acid, and the product collected and crystallised from acetic acid. Yield, 18·2 g. (96%). M. p. 180° (Found: N, 6·8; S, 15·5. $C_{21}H_{20}O_3N_2S_2$ requires N, 6·8; S, 15·4%).

p-*Benzylthiobenzaldehyde*.—The toluene-*p*-sulphonylhydrazide (18 g.) was heated in a bath at 160° with ethylene glycol (95 c.c.). The hot solution was treated with finely powdered anhydrous sodium carbonate (8·64 g.), heating being continued for 1 minute. The solution was slightly cooled and diluted with hot water (1 l.). After rapid cooling, the oil was isolated with the aid of ether; the residue after evaporation of the solvent was entirely crystalline and practically pure *aldehyde* (8 g.; 80%). For analysis a small sample was distilled under reduced pressure and crystallised from alcohol; it formed very pale-yellow leaflets, m. p. 70° (Found: C, 74·0; H, 5·6; S, 14·0. $C_{14}H_{12}OS$ requires C, 73·7; H, 5·3; S, 14·0%).

2-*Phenyl*-4-(4′-*benzylthiobenzylidene*)*oxazol*-5-*one*.—The aldehyde (8 g.), fused sodium acetate (8 g.), and hippuric acid (6·3 g.) were intimately mixed, treated with acetic anhydride (50 c.c.), and heated on the steam-bath for 15 minutes. Complete dissolution occurred before the product began to crystallise. After cooling, the product was stirred with water containing some sulphuric acid to assist the decomposition of excess acetic anhydride; the *oxazolone* was then collected, washed with water, and crystallised from acetic acid. Yield, 8·5 g. (74%) of orange prisms, m. p. 186° (Found: N, 3·9; S, 8·7. $C_{23}H_{17}O_3NS$ requires N, 3·8; S, 8·6%).

α-*Benzamido*-4-*benzylthiocinnamic Acid*.—The preceding oxazolone (14·9 g.) was added to boiling 66% alcohol (1490 c.c.) containing 1% of sodium hydroxide. The solution cleared in 1—2 minutes, and boiling was continued for about 10 minutes. Dilute hydrochloric acid was then added in excess, followed by enough water to produce turbidity. On cooling the pure *acid* separated in long prismatic needles (14·75 g.; 96%), m. p. 21—214°, unchanged by recrystallisation from 65% alcohol (Found: N, 3·9. $C_{23}H_{19}O_3NS$ requires N, 3·6%).

β-p-*Mercaptophenylalanine*.—The benzamidocinnamic acid (9·8 g.), red phosphorus (10 g.), and hydriodic acid (200 c.c., *d* 1·7) were boiled under reflux for 3 hours. After cooling the solution was filtered through asbestos, and the filtrate evaporated to dryness under reduced pressure. The dark residue immediately became colourless on treatment with water, indicating oxidation of the thiol compound by residual free iodine; the aqueous solution was therefore extracted with ether to remove benzoic acid, freed from ether by distillation, chilled in ice, and treated with 4N-iodine in potassium iodide until free iodine was present in very slight excess; the mixture was then heated, and the hot solution brought to pH 5 by addition of aqueous ammonia. The disulphido-amino-acid separated in bunches of minute needles; when cold, it was collected, washed with alcohol and ether, and dried. Yield, 4·4 g. (88%). Purification of the disulphide was difficult; it was achieved with considerable loss as follows. 1·2 G. were dissolved by warming to 50° with N-hydrochloric acid (100 c.c.), and the yellow solution was allowed to stand overnight at 15°. The gelatinous precipitate was filtered off, and the filtrate neutralised at the boiling point with concentrated ammonia solution. After several hours the solid was filtered off, washed thoroughly with water, and dried at 100°/20 mm. for analysis. Yield, 0·42 g.; m. p. 258—260° (decomp.) (Found: C, 55·0; H, 5·7; N, 7·3; S, 15·5. Calc. for $C_{18}H_{20}O_4N_2S_2$: C, 55·1; H, 5·1; N, 7·1; S, 16·3%). For the preparation of the mercapto-amino-acid, the impure disulphide was partly dissolved in boiling 5N-hydrochloric acid (10 c.c.), and zinc dust (1 g.) was added in portions to the hot solution during 10—15 minutes. The solution was filtered and mixed at the boiling point with an equal volume of concentrated hydrochloric acid. On cooling, the *hydrochloride dihydrate* separated in bunches of tiny needles which sintered and darkened at 200° and decomposed at 222° [Found: N, 5·2; S, 11·7; SH (by iodine titration) 12·2. $C_9H_{16}O_4NClS$ requires N, 5·2; S, 11·9; SH, 12·25%].

Thianaphthen-3-*aldehyde*.—(*a*) Thianaphthen-3-carboxyl chloride (Crook and Davies, *J.*, 1937, 1697; 6·3 g.) was dissolved in dry xylene (30 c.c.), 5% palladised barium sulphate (3 g.) added, and the mixture heated under reflux with vigorous stirring in a stream of hydrogen. After 4 hours, evolution of hydrogen chloride had practically ceased and the reduction was discontinued. The solution was filtered, and the filtrate shaken for 15 minutes with excess of saturated sodium bisulphite solution. The bisulphite compound was filtered off, washed with xylene, and dried on a tile. The pure aldehyde was obtained on decomposition with warm sodium carbonate in the usual way. Yield, 2·2 g. (42·5%); m. p. 54°.

(*b*) Methyl thianaphthen-3-carboxylate (Komppa and Weckmann, *loc. cit.*; 15 g.) was boiled under reflux for 8 hours with alcohol (37·5 c.c.) and hydrazine hydrate (9 c.c. of 90%). Water was then added to the solid mass, and the *thianaphthen-3-carboxyhydrazide* filtered off and crystallised from 30% methanol. It formed sheaves of needles, m. p. 176—177° (12·2 g.; 68%) (Found: C, 55·9; H, 4·3; N, 15·2. $C_9H_8ON_2S$ requires C, 56·25; H, 4·2; N, 14·6%). The hydrazide (11·3 g.) was dissolved in dry pyridine (192 c.c.), and the solution cooled to 0°. Toluene-*p*-sulphonyl chloride (11·3 g.) was then added in small portions during 15—20 minutes. After being kept at room temperature for 2 hours, the mixture was poured into 5N-HCl (465 c.c.) with stirring. The solid was collected and crystallised from 80% acetic acid. The yield of *thianaphthen-3-carboxytoluene-p-sulphonylhydrazide* was 19·2 g. (94%); m. p. 201—203°. It was dried at 110° for analysis (Found: C, 55·6; H, 4·0; N, 8·3. $C_{16}H_{14}O_3N_2S_2$ requires C, 55·5; H, 4·1; N, 8·1%). The toluene-*p*-sulphonyl derivative (3·1 g.) was dissolved in ethylene glycol (redistilled; 15 c.c.) and heated to 160°, and anhydrous sodium carbonate (0·96 g.) added in one portion. When the vigorous effervescence had ceased, heating was continued for 30 seconds, and hot water (150 c.c.) added. The aldehyde was extracted with ether and purified through the bisulphite compound as before. Yield, 0·9 g. (63%).

2-*Phenyl-4-3′-thianaphthenylmethyleneoxazol-5-one*.—The aldehyde (0·7 g.), sodium hippurate (1 g.), and acetic anhydride (2·5 c.c.) were mixed and heated at 100° for 30 minutes. The resultant yellow mass was cooled, rubbed with water, and filtered. The *oxazolone* crystallised from benzene in long yellow needles, m. p. 219—220° (Found: N, 4·8. $C_{18}H_{11}O_2NS$ requires N, 4·8%).

N-*Benzoyl-β-3-thianaphthenylalanine*.—The oxazolone (0·8 g.) was added to a mixture of pure acetic acid (4·5 c.c.), hydriodic acid (0·1 c.c.; *d* 1·7), and red phosphorus (0·2 g.) and heated under reflux for 1 hour. The solution was filtered hot, and the phosphorus washed with hot acetic acid. The *benzoyl* compound crystallised from the filtrate on cooling and addition of water. It was recrystallised from glacial acetic acid, forming hexagonal plates, m. p. 226—228° (0·7 g.; 87%) (Found: C, 66·1; H, 4·4. $C_{18}H_{15}O_3NS$ requires C, 66·4; H, 4·6%). Thianaphthen was unchanged when subjected to the above treatment, showing that under these conditions the double bond in the heterocyclic ring is not reduced.

β-3-Thianaphthenylalanine.—The benzoyl derivative (1·5 g.) was heated under reflux with a mixture of concentrated hydrochloric (50 c.c.) and glacial acetic acid (20 c.c.) for 24 hours. The light-brown solution was treated with charcoal and evaporated to dryness under reduced pressure. The residue was taken up in hot water (15 c.c.), and the solution adjusted to pH 5 with concentrated ammonia solution. The *amino-acid* separated in small plates, m. p. 248—250° (decomp.) (Found: C, 59·5; H, 5·4. $C_{11}H_{11}O_2NS$ requires C, 59·7; H, 5·0%).

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293. *Respiratory Stimulants. Part II. Fully Substituted Bisureas derived from 2 : 2′-Diaminodiethyl Ether and 1 : 3-Diamino-2-alkoxypropanes.*

By W. R. BOON.

The preparation is described of a number of diamines containing ether linkages of the type $NHR\cdot[CH_2]_n\cdot Y\cdot[CH_2]_n\cdot NHR$, where R or R′ is alkyl or alkoxyalkyl, *n* is 1 or 2, and Y is O or CH·OMe or CH·OEt. The diamines have been converted into fully substituted bisureas by reaction with dialkylcarbamyl chlorides or similar heterocyclic compounds.

IN Part I (*J.*, 1947, 307) the preparation was reported of a number of bisureas (derived from αω-alkylenediamines) of the general formula, $X\cdot CO\cdot NR\cdot[CH_2]_n\cdot NR'\cdot COY$, in which R and R′ represent alkyl or alkoxyethyl radicals and X and Y represent secondary amine residues. In the present paper the preparation of a similar series of compounds is reported, in which the central alkylene chain is interrupted by an oxygen atom or substituted by an alkoxy-group. It was thought that compounds so modified might show more useful pharmacological properties than did those reported in Part I. Apart, however, from some slight reduction in toxicity, no great advantage appeared to compensate for the greater difficulty of preparation.

The 2 : 2′-bisalkylaminodiethyl ethers required were prepared from the corresponding bisalkylanilino-compounds by hydrolysis of their nitroso-derivatives with sodium hydrogen sulphite; this procedure proved to be much less efficient than with the corresponding alkylenediamines, probably owing partly to the increased solubility of the nitrosoamine hydrochlorides and partly to the much lower volatility of the products in steam. The 2 : 2′-bisalkylanilinodiethyl ethers were first prepared, in poor yield, by condensation of 2 : 2′-dichlorodiethyl ether with the appropriate alkylaniline, the main product being 4-phenylmorpholine. During the course of this work, Brill, Webb, and Halbedel (*J. Amer. Chem. Soc.*, 1941, **63**, 971) reported the isolation only of the morpholine under similar conditions. The best method of preparing the

required ethers was condensation of an alkyl-2-chloroethylaniline with the sodium derivative of an alkyl-2-hydroxyethylaniline, this having the further advantage that it was available for the preparation of unsymmetrical diamines. *Methyl* 1 : 3-*bismethylamino-2-propyl ether* was prepared in this way from *methyl* 1 : 3-*bismethylanilino-2-propyl ether*, itself obtained from the corresponding *propanol*; a more convenient method was direct interaction of methyl 1 : 3-dichloro-2-propyl ether with excess of methylamine; *ethyl* 1 : 3-*bis*-2′-n-*butoxyethylamino-2-propyl ether* was prepared by procedure analogous to this last method.

An alternative method for the preparation of methyl 1 : 3-bismethylamino-2-propyl ether was examined, involving the hydrolysis of its *bistoluene*-p-*sulphonyl* derivative. It was hoped that this compound could be hydrolysed under relatively mild conditions, thus avoiding the danger of possible hydrolysis of the ether group inherent in the normal methods for hydrolysing toluene-*p*-sulphonamides; in addition all products of the reaction should be volatile, thus simplifying isolation of the diamine which is only slightly volatile in steam. The toluenesulphonamide was unaffected by being heated with constant-boiling hydrochloric acid, and this line was not pursued further.

All the amines were converted smoothly into the ureas by reaction with the appropriate carbamyl chloride.

Experimental.

(Preparations marked by an asterisk were carried out by Dr. J. A. Hendry. Microanalyses are by Mr. E. S. Morton.)

2 : 2′-*Bismethylanilinodiethyl Ether*.—Of several conditions examined the following gave the best yield. 2 : 2′-Dichlorodiethyl ether (117 g., 0·8 mol.), methylaniline (357 g., 3·35 mols.), anhydrous potassium carbonate (170 g.), and copper powder (2 g.) were stirred for 14 hours at 100—105°. Water was added, and the oil separated and distilled under reduced pressure, giving 235 g. of material, largely recovered methylaniline, boiling below 130°/16 mm., 75 g. (55·2%) of phenylmorpholine, b. p. 158—160°/22 mm., m. p. 53° (from methanol), and 63 g. (36·3%) of 2 : 2′-*bismethylanilinodiethyl ether*, b. p. 258—260°/22 mm., m. p. 45° (from methanol) (Found: N, 10·3. $C_{18}H_{42}ON_2$ requires N, 9·9%).

2 : 2′-*Bisethylanilinodiethyl ether* was obtained similarly in 13% yield, b. p. 255—256°/20 mm., m. p. 37° (from methanol) (Found: N, 9·1. $C_{20}H_{26}ON_2$ requires N, 9·0%). This compound was also prepared as follows. Ethylhydroxyethylaniline (375 g., 2·3 mols.) was dissolved in toluene (250 c.c.), and dried by distillation. After the solution had been cooled to 30°, sodium (45 g., 1·96 atoms) was added and, after the reaction had subsided, the whole was stirred under reflux for 3 hours. Ethylchloroethylaniline (350 g., 1·9 mols.) was then added, and heating continued under reflux with stirring for 15 hours. After being cooled, the mixture was washed twice with water (500 c.c. in all) and distilled under reduced pressure. Yield, 444 g. (75%).

2-*Methylanilino*-2′-*ethylanilinodiethyl ether* was prepared by the second method described above, from methyl-2-hydroxyethylaniline and ethyl-2-chloroethylaniline in 73% yield; b. p. 248—250°/13 mm. (Found: C, 76·0; H, 8·9; N, 9·6. $C_{19}H_{26}ON_2$ requires C, 76·5; H, 8·7; N, 9·4%).

1 : 3-*Bismethylanilino*-2-*propanol*.—Dichlorohydrin (903 g., 7 mols.), methylaniline (1926 g., 18 mols.), and powdered calcium carbonate were stirred at 150° for 45 hours. After the mixture had been cooled to 80°, water was added and the whole filtered through a steam-jacketed Buchner funnel. After separation of the water, the oil was distilled giving 1420 g. (75%) of 1 : 3-*bismethylanilino*-2-*propanol*, b. p. 220°/0·25 mm., m. p. 80° (from light petroleum, b. p. 60—80°) (Found: C, 75·3; H, 8·6; N, 10·5. $C_{17}H_{22}ON_2$ requires C, 75·4; H, 8·2; N, 10·4%).

Methyl-1 : 3-*bismethylanilino*-2-*propyl Ether*.—Sodium (46 g.) was added to a solution of 1 : 3-bismethylanilino-2-propanol (540 g., 2 mols.) in xylene (1 l.). After the reaction had subsided, the mixture was heated under reflux until all the sodium had dissolved. After the mixture had been cooled to 25°, methyl sulphate (252 g., 2 mols.) was added in one lot, whereupon the temperature rose to 120°. After the temperature had fallen below 100°, the whole was stirred under reflux for 10 hours. After cooling it, water was added, followed by sodium carbonate until the mixture was alkaline to brilliant-yellow. The xylene layer was separated and extracted with acid. The acid layer was basified with sodium hydroxide and extracted with benzene. Distillation of the benzene solution gave 253 g. (45%) of *methyl* 1 : 3-*bismethylanilino*-2-*propyl ether*, b. p. 185°/0·5 mm. (Found: C, 75·7; H, 8·8; N, 10·3. $C_{18}H_{24}ON_2$ requires C, 76·0; H, 8·6; N, 9·9%).

1 : 3-*Bistoluene*-p-*sulphonylmethylamido*-2-*propanol*.—Sodium (8·7 g., 0·378 atom) was broken up in hot xylene (350 c.c.), and ethanol (35 c.c.) was added, reaction being completed by stirring under reflux. After addition of toluene-p-sulphonylmethylamide (70 g., 0·378 mol.), the ethanol was distilled off, dichlorohydrin (24 g., 0·186 mol.) was added, and heating continued under reflux with stirring for 20 hours. The xylene was then removed by steam-distillation, the residue basified with sodium hydroxide, and the aqueous layer separated. The residual oil was triturated with methanol, and the resultant solid was filtered off and recrystallised from methanol, giving 48 g. (62%) of 1 : 3-*bistoluene*-p-*sulphonylmethylamido*-2-*propanol*, m. p. 118° (Found: C, 53·4; H, 5·7; N, 6·5. $C_{18}H_{24}O_5N_2S_2$ requires C, 53·4; H, 6·1; N, 6·6%).

Methyl 1 : 3-*Bistoluene*-p-*sulphonmethylamido*-2-*propyl Ether*.—To 1 : 3-bistoluene-*p*-sulphonylmethylamido-2-propanol (43 g., 0·1 mol.) in toluene (300 c.c.), sodium (2·3 g.) was added, and the whole heated under reflux until the sodium was dissolved. After cooling, methyl sulphate (13 g., 0·103 mol.) was added, whereupon the temperature rose to 40°. After 48 hours the toluene was removed by distillation in steam, and the residue crystallised twice from methanol. However, the product had an indefinite

m. p. and was further purified by crystallisation from carbon tetrachloride, which gave 10 g. of unchanged starting material. The residue obtained on evaporation of the mother-liquor gave, on crystallisation from methanol, 10 g. (25%) of *methyl* 1 : 3-*bistoluene*-p-*sulphonmethylamido*-2-*propyl ether*, m. p. 88° (mixed m. p. with starting material 76°) (Found: C, 54·9; H, 7·0; N, 6·3. $C_{29}H_{36}O_5N_4$ requires C, 54·6; H, 6·4; N, 6·4%).

Bisureas, $(X{\cdot}CO{\cdot}NR{\cdot}[CH_2]_n)_2Y$.

				Yield,			Analysis. Found, %.			Required, %.		
X.	R.	Y.	*n*.	%.	B. p.	Formula.	C.	H.	N.	C.	H.	N.
Me_2N	Me	O	2	96	254°/ 30 mm.	$C_{12}H_{26}O_3N_4$	52·3	9·2	20·5	52·6	9·5	20·5
Et_2N	Me	O	2	93	244/ 12 mm.	$C_{16}H_{34}O_3N_4$	58·4	9·9	16·7	58·2	10·3	16·9
$[CH_2]_5>N$	Me	O	2	68·5	286/ 14 mm.	$C_{18}H_{34}O_3N_4$	60·9	9·5	16·1	61·0	9·6	15·8
$O<[CH_2]_4>N$	Me	O	2	86	262/ 0·8 mm.	$C_{16}H_{30}O_5N_4$	53·4	8·1	—	53·6	8·4	—
Et_2N	Et	O	2	89	239/ 18 mm.	$C_{18}H_{38}O_3N_4$	61·1	10·6	15·7	60·9	10·6	15·7
$O<[CH_2]_4>N$	Et	O	2	74	228/ 0·3 mm.	$C_{18}H_{34}O_5N_4$	55·6	8·7	14·3	55·8	8·8	14·5
Et_2N	Me	CH·OMe	1	69	228/ 21 mm.	$C_{16}H_{34}O_5N_4$	57·8	9·8	17·2	58·1	10·3	17·0
$[CH_2]_5>N$	Me	CH·OMe	1	80	265/ 15 mm.	$C_{18}H_{34}O_3N_4$	60·6	9·6	15·9	61·0	9·7	15·8
$O<[CH_2]_4>N$	$[CH_2]_2{\cdot}OBu^n$	CH·OEt	1	40	256/ 0·4 mm.	$C_{27}H_{52}O_7N_4$	59·2	9·6	10·0	59·5	9·6	10·3

The following di-secondary amines were prepared from the corresponding anilino-derivatives by the general method given in Part I (*loc. cit.*, p. 313): 2 : 2′-bismethylaminodiethyl ether, b. p. 180—182° [*picrate*, m. p. 87° (from water) (Found: C, 36·9; H, 3·7; N, 19·3. $C_{18}H_{32}O_{15}N_8$ requires C, 36·6; H, 3·7; N, 19·0%)]; 2-methylamino-2′-ethylaminodiethyl ether, b. p. 196—197° (no salt satisfactory for analysis was prepared); 2 : 2′-bisethylaminodiethyl ether, b. p. 204° [*picrate* (from water), m. p. 125—126° (Found: C, 38·9; H, 4·2; N, 18·0. $C_{20}H_{36}O_{14}N_8$ requires C, 38·9; H, 4·3; N, 18·2%)]. Methyl 1 : 3-bismethylamino-2-propyl ether, b. p. 175° [*picrate* (from water), m. p. 169° (Found: C, 36·7; H, 3·7; N, 19·1. $C_{18}H_{32}O_{15}N_8$ requires C, 36·6; H, 3·7; N, 19·0%). The last compound was also prepared as follows. Methyl 1 : 3-dichloro-2-propyl ether (Hess and Fink, *Ber.*, 1915, **48**, 2003) (143 g., 1 mol.) and 20% aqueous methylamine (1400 g., 9·3 mols.) were heated in an autoclave at 100° for 12 hours and then at 105—110° for a further 36 hours. The mixture was made alkaline to Clayton-yellow with sodium hydroxide, and the excess of methylamine distilled off. After acidification, the residue was concentrated to 400 c.c., filtered, basified with sodium hydroxide, and distilled to dryness under reduced pressure. Sodium hydroxide (400 g.) was added to the distillate, and the amine layer was separated, dried (KOH), and distilled; b. p. 176—179° yield; 60 g. (45·5%).

Ethyl 1 : 3-bis-2′-*n*-butoxyethylamino-2-propyl ether, b. p. 197—199°/12 mm. [*dihydrochloride*, m. p. 274—275° (Found: C, 52·3; H, 10·0; N, 7·0. $C_{17}H_{38}O_3N_2,2HCl$ requires C, 52·2; H, 10·2; N, 7·2%)], was obtained in 52% yield by the method last described.

The symmetrical bisureas listed in the Table were made by treating equimolecular proportions of a diamine with the appropriate carbamyl chloride in an inert solvent; usually the reaction was complete at room temperature, but in a few cases heating on the steam-bath for 3—5 hours was required. The diamine hydrochloride was then filtered off, and the urea isolated by shaking the filtrate with a saturated solution of potassium carbonate, drying (K_2CO_3), and distilling.

2-*Methylamino*-2′-*ethylaminodiethyl ether* NN′-*di*(*carboxydiethyl*)*amide*, prepared in 91% yield by the above method from 2-methylamino-2′-ethylaminodiethyl ether and diethylcarbamyl chloride, had b. p. 232°/15 mm. (Found: C, 59·6; H, 10·5; N, 16·3. $C_{17}H_{36}O_3N_4$ requires C, 59·2; H, 10·5; N, 16·3%).

N-*Methyl*-N′N′-*diethyl*-N-(3-*methylamino*-2-*methoxypropyl*)*urea*.—Diethylcarbamyl chloride (22 g., 0·16 mol.) in toluene (50 c.c.) was added, with stirring, to methyl 1 : 3-bismethylamino-2-propyl ether (32 g., 0·24 mol.) in toluene (150 c.c.) at −5°. After 16 hours at room temperature, the amine hydrochloride was removed by filtration, and N-*methyl*-N′N′-*diethyl*-N-(3-*methylamino*-2-*methoxypropyl*)*urea* isolated by distillation; yield, 13 g. (35%); b. p. 150—152°/12 mm. (Found: C, 57·1; H, 10·3; N, 18·0. $C_{11}H_{25}O_2N_3$ requires C, 57·2; H, 10·2; N, 18·2%). 6 G. of the bisurea, b. p. 210°/12 mm., were also obtained.

Methyl 1 : 3-*Bismethylamino*-2-*propyl Ether* N-*Carboxydiethylamide* N′-*Carboxypiperidide*.—*N*-Methyl-*N*′*N*′-diethyl-*N*-(3-methylamino-2-methoxypropyl)urea (13 g., 0·56 mol.) and piperidine-1-carboxyl chloride (4·5 g., 0·33 mol.) were mixed, and, after the initial heat evolution had ceased, were heated at 100° for 1 hour. Saturated potassium carbonate solution (50 c.c.) was then added, and the whole heated under reflux for 10 minutes. After the mixture had been cooled, the oil was extracted with ether, and the extract dried (K_2CO_3) and distilled, giving 7 g. (72%) of the *bisurea*, b. p. 228°/8 mm. (Found: C, 59·3; H, 10·2; N, 16·2. $C_{17}H_{34}O_3N_4$ requires C, 59·7; H, 9·9; N, 16·4%).

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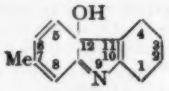
294. *The Fischer Indole Synthesis. Part I.*

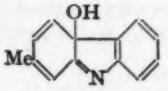
By C. S. Barnes, K. H. Pausacker, and C. I. Schubert.

The cyclisation of a number of *o*-substituted phenylhydrazones of *cyclo*hexanone has been studied, and the structure of the by-products (termed 1 : 2 : 3 : 4-tetrahydro*iso*carbazoles) postulated.

In a previous communication (Pausacker and Robinson, *J.*, 1947, 1557) it was noted that two products were produced by the action of dilute sulphuric acid on the 2-chloro-5-methylphenylhydrazone of *cyclo*hexanone. The first product was the expected 8-chloro-5-methyl-1 : 2 : 3 : 4-tetrahydrocarbazole and the other, formed in small yield, was assigned the structure (I). On dehydrogenation, (I) yielded a product which was considered to have structure (II). It is now proposed that these be called respectively 12-hydroxy-7-methyl-1 : 2 : 3 : 4-tetrahydro- and 12-hydroxy-7-methyl-*iso*carbazole.



(I.)



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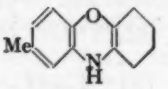
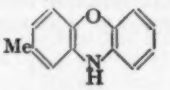


(III.)



(IV.)

It was suggested that these compounds might possibly have the phenoxazine structures (III and IV) but this was considered unlikely, as their melting points appeared to be too high.

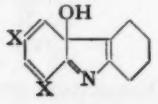
Complete proof that these compounds are not of the phenoxazine type was obtained when 12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* (V), m. p. 170°, was formed, in small amount, during the cyclisation of the *o*-chlorophenylhydrazone of *cyclo*hexanone by dilute sulphuric acid. Neither the same by-product nor its acetyl derivative was formed when glacial acetic acid was the cyclising agent. It was readily separated from the major product, 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole, owing to its sparing solubility in light petroleum (b. p. 40—60°). It was devoid of phenolic properties (*e.g.*, no colour with ferric chloride and insoluble in sodium hydroxide solution) and formed both a *picrate* and an *acetyl* derivative. On de hydrogenation with palladised charcoal, 12-*hydroxyisocarbazole* (VI), m. p. 267°, was formed. Phenoxazine has m. p. 156°, so that it is obvious that (VI) cannot be identical with phenoxazine and so the original hypothesis has been confirmed.

Although (V) and (VI) do not display phenolic properties, it is still possible that (V) may be 8-hydroxy-1 : 2 : 3 : 4-tetrahydrocarbazole, formed by hydrolysis of 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole, and (VI) may be 1-hydroxycarbazole. This supposes that the phenolic properties are masked in some way by the neighbouring nitrogen atom. Accordingly, 1-hydroxycarbazole (VII) was synthesised as follows. 2-Methoxyphenylhydrazine (Charrier and Casale, *Gazzetta*, 1914, **44**, I, 617; cf. Beilstein, vol. 15, 187) was condensed with *cyclo*hexanone and the corresponding *hydrazone* was cyclised with dilute sulphuric acid. The products formed in this reaction were separated into two fractions by extraction with ether. The ether-insoluble portion was a compound, m. p. 145—146°, assumed to be 12-*methoxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole*, whilst the ether-soluble portion was 8-*methoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole* (VIII). On dehydrogenation of (VIII) with chloranil (cf. Barclay and Campbell, *J.*, 1945, 530), 1-*methoxycarbazole* (IX) resulted. When palladised charcoal was used as the dehydrogenating agent, a mixture of (IX) and carbazole was formed. On hydrolysis of (IX) with concentrated hydrobromic acid/glacial acetic acid (cf. Stoermer, *Ber.*, 1908, **41**, 323), 1-*hydroxycarbazole* (VII), m. p. 160°, was isolated. It was found to be a typical phenol, dissolving in dilute sodium hydroxide solution and giving a green colour with ferric chloride solution, so it cannot be identical with (VI). A prior claim (D.R.-P. 258,298; Frl., **11**, 170) to the preparation of (VII) has been made, and it was stated to have m. p. 163°. This method of preparation involved the acid hydrolysis of 1-hydroxycarbazole-3 : 6-disulphonic acid at elevated temperature. The latter compound had been prepared by the fusion of carbazole-1 : 3 : 6-trisulphonic acid with sodium hydroxide. As the structure of these sulphonic acids is not known with certainty, it is believed that the above synthesis offers an unambiguous approach to the preparation of (VII).

Similarly, *o*-phenetidine was converted into *o*-ethoxyphenylhydrazine, and on cyclisation with dilute sulphuric acid both 8-*ethoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole* (X) and 12-*ethoxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* were isolated. After the dehydrogenation of (X) with bromanil,

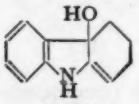
which has been found to be as efficacious as chloranil, 1-*ethoxycarbazole* was formed, which also yielded (VII) upon hydrolysis.

It is now apparent that *o*-halogeno- and *o*-alkoxy-phenylhydrazones of *cyclo*hexanone tend to form both the appropriate tetrahydrocarbazole and tetrahydro*iso*carbazole. Now, if a 2 : 6-substituted phenylhydrazone of *cyclo*hexanone were cyclised, then tetrahydrocarbazole formation would be prevented and tetrahydro*iso*carbazole should be the only product of reaction. This conclusion was verified by examining the cyclisation of the 2 : 4 : 6-trichloro- and 2 : 4 : 6-tribromo-phenylhydrazones of *cyclo*hexanone, whereby (XI) and (XII) respectively were obtained, to which formulæ (XI) and (XII) are assigned. These compounds both formed *picrates* and (XII) also formed an *acetyl* derivative. On dehydrogenation with palladised charcoal in the presence of hydrogen, both compounds were converted into carbazole, thus demonstrating the nature of the fundamental skeleton. Under the same conditions (I) and (V) are simply dehydrogenated to form (II) and (VI) respectively. This difference in reactivity is attributed to the fact that (XI) and (XII) are first dehalogenated, and the hydrogen halide formed then converts the hydroxyl into halogen. Carbazole is then formed by subsequent dehalogenation and dehydrogenation. Furthermore, (XII) was dehydrogenated with bromanil to form 6 : 8-*dibromo*-12-*hydroxy*iso*carbazole* (isolated as its *picrate*).

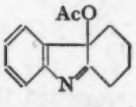


(XI; X = Cl.)

(XII; X = Br.)



(XIII.)



(XIV.)

An attempt is being made to convert either 11-hydroxy-2 : 3 : 4 : 11-tetrahydrocarbazole (XIII) (Plant and Tomlinson, *J.*, 1931, 3330) or 11-acetoxy-1 : 2 : 3 : 4-tetrahydrocarbazolenine (XIV) (Perkin and Plant, *J.*, 1923, **123**, 688) into 12-hydroxy*iso*carbazole (VI).

In many cases the corresponding amine ($Ar{\cdot}NH_2$) has been isolated from the products formed during the cyclisation of the hydrazone $\left(Ar{\cdot}NH{\cdot}N{:}C\begin{<}\begin{matrix}CH_2{-}CH_2\\CH_2{-}CH_2\end{matrix}\begin{>}CH_2\right)$.

Further work is proceeding upon the structure of the tetrahydro*iso*carbazoles, and their possible significance in elucidating the mechanism of the Fischer indole synthesis is outlined in the following paper.

Experimental.

(M. p.s are not corrected. Analyses by Drs. Weiler and Strauss, Dr. Tettweiler, and Miss D. Wauchope.)

8-*Methoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole*.—2-Methoxyphenylhydrazine (Charrier and Casale, *loc. cit.*) was converted into *cyclo*hexanone 2-methoxyphenylhydrazone (XV) in the usual manner. It crystallised from ether as long white needles, m. p. 67—68°, but owing to its instability, it was not analysed. It (68 g.) was added to a mixture of concentrated sulphuric acid (65 ml.) and water (590 ml.) preheated to 90°. After initial reaction, the mixture was heated under reflux (5 minutes). This process is subsequently referred to as " sulphuric acid cyclisation." The resulting mixture was extracted with ether and the ether-insoluble fraction was filtered off and washed with ether. From the combined ethereal extracts, 8-*methoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole* (XVI) was obtained, b. p. 205—210°/15 mm. (Found : C, 77·3; H, 7·5. $C_{13}H_{15}ON$ requires C, 77·7; H, 7·5%). A very small amount of carbazole (m. p. and mixed m. p. with an authentic specimen, 243°) was also isolated from a high-boiling fraction. *o*-Anisidine (2·8 g.) (identified by its acetyl derivative and picrate) was obtained upon basification of the aqueous portion.

When a concentrated benzene solution of the amorphous ether-insoluble product (11 g.) was allowed to stand in the refrigerator, white crystals (m. p. 137—144°) separated. After crystallisation from light petroleum (b. p. 60—90°), 12-*methoxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* was obtained as small prisms, m. p. 145—146° (Found : N, 7·0. $C_{13}H_{15}ON$ requires N, 7·0%). It was soluble in concentrated hydrochloric acid, but reprecipitated on addition of water.

Similarly, (XV) (24 g.) was heated under reflux (8 minutes) with glacial acetic acid (250 ml.). The oil precipitated on dilution was immediately extracted with ether and, from the dried ethereal extract, (XVI) (10·4 g.) was again obtained, b. p. 207—210°/14 mm. (Found : C, 77·6; H, 7·3%). *o*-Anisidine (*ca.* 2 g.) was also obtained from the low-boiling fraction.

1-*Methoxycarbazole*.—The compound (XVI) (4·1 g.) was heated under reflux (0·5 hour) with chloranil (10 g.) in sulphur-free xylene (cf. Barclay and Campbell, *loc. cit.*). After filtration, the filtrate was extracted with dilute sodium hydroxide solution, washed with water, and the xylene evaporated under reduced pressure. The residue was dissolved in benzene and chromatographed on an alumina column. That portion of the column giving a blue fluorescence in ultra-violet light was extruded and extracted with acetone; 1-*methoxycarbazole* (XVII) (2·0 g.) was obtained, and crystallised from aqueous ethyl alcohol as long white needles, m. p. 69—70° (Found : C, 79·0; H, 6·0. $C_{13}H_{11}ON$ requires C, 79·1; H, 5·6%). The *picrate* crystallised from aqueous alcohol as orange needles, m. p. 144—145° (Found : C, 53·8; H, 3·4. $C_{19}H_{14}O_8N_4$ requires C, 53·5; H, 3·3%). When (XVI) was dehydrogenated by palladised charcoal in a stream of hydrogen (300°), a mixture of (XVII) and carbazole was obtained.

1-*Hydroxycarbazole*.—1-Methoxycarbazole (0·9 g.) was heated under reflux (2 hours) with glacial acetic acid (10 ml.) and concentrated hydrobromic acid (2 ml.) in an atmosphere of carbon dioxide (cf. Stoermer, *loc. cit.*). The solution was diluted with water, extracted with ether, and the ethereal extract washed, first with sodium hydrogen carbonate solution, and then with water. From the dried ethereal solution, crude 1-*hydroxycarbazole* (XVIII) (0·8 g.) was obtained; it crystallised from water (charcoal) as long white needles, m. p. 160° (Found: C, 79·3; H, 5·3; N, 7·4. $C_{12}H_9ON$ requires C, 78·7; H, 4·95; N, 7·65%). (XVIII) rapidly darkened on exposure to light (cf. 3-hydroxycarbazole), dissolved readily in sodium hydroxide solution, and gave a green colour with ferric chloride solution. When it was dissolved in concentrated sulphuric acid, and a drop of concentrated nitric acid added, a brown colour developed. Carbazole and many of its simple derivatives produced a deep ultramarine-blue colour in this test although exceptions have been noted (Barclay and Campbell, *loc. cit.*). Its *picrate*, which was apparently quite stable, crystallised from water as orange needles, m. p. 191—192° (Found: C, 52·6; H, 3·3. $C_{18}H_{12}O_8N_4$ requires C, 52·4; H, 3·0%). The *acetyl* derivative formed small white prisms, m. p. 132—133°, from water (Found: N, 6·5. $C_{14}H_{11}O_2N$ requires N, 6·7%).

8-*Ethoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole*.—*o*-Nitrophenetole was prepared from *o*-nitrophenol by van Erp's method (*Ber.*, 1923, **56**, 217) and reduced to *o*-phenetidine by zinc dust and sulphuric acid (cf. Beilstein, Vol. 13, p. 359). *o*-Phenetidine (23 g.) was converted into the corresponding hydrazine by essentially the same method as used by Charrier and Casale (*loc. cit.*) for the preparation of *o*-methoxyphenylhydrazine from *o*-anisidine (cf. also Franzen and Schmidt, *J. pr. Chem.*, 1917, **96**, 17, for the preparation of the hydrazine). The crude hydrazine (18 g.) was then condensed with *cyclo*hexanone, and the resulting hydrazone cyclised with dilute sulphuric acid, the ethereal extract yielding 8-*ethoxy*-1 : 2 : 3 : 4-*tetrahydrocarbazole* (XIX) (10·3 g.), b. p. 180—186°/3 mm. The analysed product had b. p. 172—173°/2 mm. (Found: N, 6·5. $C_{14}H_{17}ON$ requires N, 6·5%). It was later found to crystallise from light petroleum (b. p. 40—60°) as colourless needles, m. p. 75—76°. The *picrate* formed dark red needles from benzene, m. p. 179° (Found: N, 12·5. $C_{20}H_{20}O_8N_4$ requires N, 12·6%). In addition, a tarry product was formed, which was insoluble in both ether and the dilute sulphuric acid. This was dissolved in alcohol, the solution concentrated, and the solid (1·6 g.) formed by the addition of water was centrifuged. After solution in benzene (charcoal), small off-white prisms were formed on evaporation in the air. This compound is assumed to be 12-*ethoxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole*, m. p. 202—204° (from light petroleum, b. p. 100—120°) (Found: N, 6·7. $C_{14}H_{17}ON$ requires N, 6·5%).

1-*Ethoxycarbazole*.—The tetrahydrocarbazole (XIX) (4·3 g.) was heated under reflux (14 hours) with bromanil (17 g.) in sulphur-free xylene (200 ml.). After separation of the tetrabromoquinol, the solution was washed with dilute sodium hydroxide solution and several times with water. After distillation, crude 1-*ethoxycarbazole* (XX) was isolated (3·4 g., b. p. 185—200°/2·4 mm.). Purification through its picrate yielded the pure substance, m. p. 95°, crystallising in white needles from light petroleum (b. p. 60—90°) (Found: N, 6·7. $C_{14}H_{13}ON$ requires N, 6·6%); *picrate* (red needles crystallising from benzene), m. p. 171° (Found: N, 13·1. $C_{20}H_{16}O_8N_4$ requires N, 12·7%). Hydrolysis with hydrobromic acid–acetic acid yielded 1-hydroxycarbazole, identical with the product from the hydrolysis of 1-methoxycarbazole.

12-*Hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole*.—The *o*-chlorophenylhydrazone of *cyclo*hexanone (57 g.) was cyclised with dilute sulphuric acid, and the resulting dark oil extracted with ether. A light purple solid (1·3 g.) was insoluble in both the ether and dilute sulphuric acid. This compound has not been investigated further. Distillation of the ethereal extract gave a fraction (35·2 g., b. p. 160—180°/4 mm.) which deposited a white solid (1·7 g.) upon the addition of light petroleum (b. p. 40—60°). The major product, *i.e.*, the known 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole, remained in solution. The solid was 12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* (XXI), crystallising from light petroleum (b. p. 100—120°) as white needles, m. p. 172° [Found: C, 77·0; H, 6·9; N, 7·3; *M* (Rast), 183. $C_{12}H_{13}ON$ requires C, 77·0; H, 7·0; N, 7·5%; *M*, 187]. A further quantity was obtained from the residue of the above distillation; *picrate* (microscopic dark-red needles crystallising from benzene), m. p. 166° (Found: C, 51·4; H, 3·8; N, 13·4. $C_{18}H_{16}O_8N_4$ requires C, 51·9; H, 3·9; N, 13·4%); *acetyl* derivative (white needles from light petroleum, b. p. 100—120°), m. p. 135—136° (Found: N, 6·2. $C_{14}H_{15}ON$ requires N, 6·1%). The original dilute sulphuric acid solution gave a positive test for chloride ion, and after basification, yielded a small amount of *o*-chloroaniline (identified as acetyl derivative). The substance (XXI) was insoluble in dilute sodium hydroxide solution, gave a negative ferric chloride colour, and its benzene solution showed a strong blue fluorescence in ultraviolet light.

When glacial acetic acid was used as the cyclising agent, 8-chloro-1 : 2 : 3 : 4-tetrahydrocarbazole was again isolated in good yield, but neither (XXI) nor its acetyl derivative could be detected. The only by-product isolated was the *acetyl* derivative of *o*-chlorophenylhydrazine, crystallising from light petroleum (b. p. 100—120°) as white needles, m. p. 119—120° (Found: N, 14·9. $C_8H_9ON_2Cl$ requires N, 15·2%).

Dehydrogenation of (XXI) (0·5 g.) with palladised charcoal (0·2 g., 3 hours at 280—300°) gave 12-*hydroxyisocarbazole* (0·3 g.), crystallising from light petroleum (b. p. 100—120°) as white plates, m. p. 267° (Found: N, 7·6. $C_{12}H_9ON$ requires N, 7·7%).

6 : 8-*Dibromo*-12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole*.—*s*-Tribromoaniline was converted into 2 : 4 : 6-tribromophenylhydrazine (Neufeld, *Annalen*, 1888, **248**, 96); the dry hydrazine was heated (2 hours) on the water-bath under reduced pressure with a slight excess of *cyclo*hexanone, and the *hydrazone* (XXII) thus formed crystallised from alcohol as white needles, m. p. 74° (Found: C, 33·6; H, 3·25. $C_{12}H_{13}N_2Br_3$ requires C, 33·9; H, 3·1%).

(*a*) *Cyclisation with dilute sulphuric acid.* The hydrazone (XXII) (71 g.) was heated under reflux (0·5 hour) with sulphuric acid (50 ml. diluted with 450 ml. water). Steam-distillation yielded crude *s*-tribromobenzene (16 g.), crystallising from ethyl alcohol as long white needles, m. p. and mixed m. p. with an authentic specimen 122° (Found: C, 23·2; H, 1·3; Br, 75·7. Calc. for $C_6H_3Br_3$: C, 22·9; H, 1·0; Br, 76·1%). The exact origin of this substance is in doubt although it is known (Chattaway and Irving, *J.*, 1931, 1741) that *s*-trichlorophenylhydrazine is readily oxidised to *s*-trichlorobenzene. The residue was extracted with ether (bromide ion was detected in the aqueous solution) and, after

evaporation, a brown oil (40 g.) remained. This was heated under reflux with benzene, and after concentration (to *ca.* 75 ml.), light petroleum (b. p. 60—90°) was added. The dirty solid (17·3 g.) precipitated was discarded, and the filtrate evaporated to dryness and then dissolved in light petroleum (b. p. 60—90°). After being left overnight in the refrigerator, crystals (6·9 g.) had separated which proved to be identical with *s*-tribromoaniline (m. p. and mixed m. p. with an authentic specimen, 122°). After evaporation of the light petroleum a dark oil (12·3 g.) remained. This was heated with picric acid (8·3 g.) in benzene; 6 : 8-*dibromo*-12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* (as XII) *picrate* (14 g.) was obtained, m. p. 142—144°, which after crystallisation from light petroleum (b. p. 60—90°) formed orange needles, m. p. 144—145° (Found: N, 10·1. $C_{18}H_{14}O_8N_4Br_2$ requires N, 9·8%). Decomposition of the picrate with dilute sodium hydroxide solution gave the *carbazole* (XII), crystallising from ethyl alcohol as thick rods, m. p. 93—94° (Found: N, 4·2. $C_{12}H_{11}ONBr_2$ requires N, 4·1%). Its *acetyl* derivative was prepared by heating (water-bath) with acetic anhydride containing a trace of concentrated sulphuric acid. It crystallised from methyl alcohol in fine white needles, m. p. 139—140° (Found: N, 3·7. $C_{14}H_{13}O_2NBr_2$ requires N, 3·6%).

After dehydrogenation (4 hours, 300°) of (XII) (0·5 g.) mixed with palladised charcoal (0·15 g.) in a stream of hydrogen, carbazole (0·07 g.) was isolated, m. p. and mixed m. p. with an authentic specimen 243° (Found: N, 8·2. Calc. for $C_{12}H_9N$: N, 8·4%).

The carbazole (XII) (1 g.) was refluxed (41 hours) with bromanil (2·46 g.) in sulphur-free xylene (30 ml.). After separation of the tetrabromoquinol, etc. (see above), a brown oil (0·7 g.) resulted. This was treated with picric acid (0·7 g.) in benzene, and the *picrate* of 6 : 8-*dibromo*-12-*hydroxyisocarbazole* was obtained, crystallising from light petroleum (b. p. 100—120°) as orange needles, m. p. 118° (Found: N, 10·3. $C_{18}H_{10}O_8N_4Br_2$ requires N, 9·8%).

(*b*) *Cyclisation with glacial acetic acid.* The hydrazone (XXII) (83 g.) was heated under reflux (0·5 hour) with glacial acetic acid (450 ml.), a dark brown solution being obtained. After separation of the crystals precipitated during the reaction (10 g., found to be ammonium bromide), the solution was concentrated in a vacuum on the water-bath. Distillation of the residue gave the following fractions: (i) <130°/3 mm. (3·2 g.); (ii) 130—190°/0·8 mm. (10·7 g.); (iii) 190—210°/0·8 mm. (11·3 g.). Fraction (i) was found to consist principally of *s*-tribromobenzene, and fraction (ii) to be *s*-tribromoaniline. Fraction (iii), after purification through its picrate, yielded (XII) (6·8 g.), identical with the product formed in method (*a*).

6 : 8-*Dichloro*-12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole*.—2 : 4 : 6-Trichlorophenylhydrazine (prepared according to the method given by Chattaway and Irving, *loc. cit.*) was condensed with *cyclo*hexanone; the phenylhydrazone (XXIII) crystallised from methyl alcohol as white needles, m.p. 72°, which decomposed on standing. It (28 g.) was heated under reflux (1 hour) in glacial acetic acid (200 ml.). The precipitated solid (ammonium chloride) was filtered off, and the glacial acetic acid was removed in a vacuum. On steam-distillation, *s*-trichloroaniline (3·8 g.) was obtained (m. p. and mixed m. p. with an authentic specimen 77°). The residue was extracted with ether and, after distillation, yielded crude 6 : 8-*dichloro*-12-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroisocarbazole* (XI) (7·8 g.), b. p. 196—206°/0·6 mm.; on redistillation, b. p. 188—190°/0·3 mm. (Found: N, 6·2. $C_{12}H_{11}ONCl_2$ requires N, 5·5%); the *picrate* formed fine copper-coloured needles [from light petroleum (b. p. 60—90°)], m. p. 114—115° (Found: N, 12·0. $C_{18}H_{14}O_8N_4Cl_2$ requires N, 11·6%).

When the base (1·2 g.) was heated (5·5 hours at 280—300°) with palladised charcoal (0·3 g.) in a stream of hydrogen until the evolution of hydrogen chloride was complete, the product extracted with acetone, and the residue sublimed at 170—180°/0·3 mm., crystallisation from light petroleum (b. p. 100—120°) afforded pure carbazole (m. p. and mixed m. p. with an authentic specimen, 240°).

One of us (K. H. P.) thanks Professor Sir Robert Robinson, P.R.S., and Dr. S. G. P. Plant for helpful discussion, the University of Melbourne for a travelling scholarship, and the Carnegie Trust for a grant when part of this work was performed. In addition, one of us (C. S. B.) thanks the Commonwealth Research Fund for financial assistance.

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295. *The Fischer Indole Synthesis. Part II.*

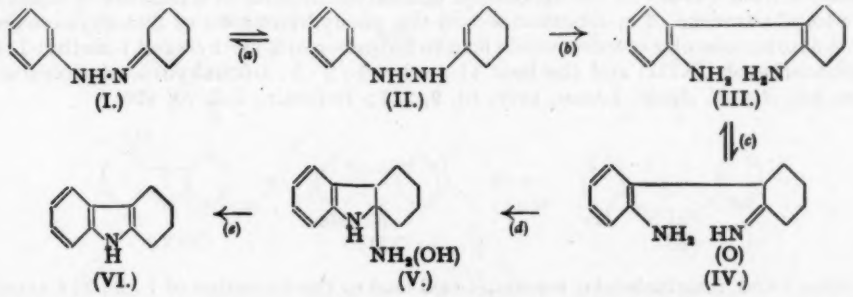
By K. H. Pausacker and C. I. Schubert.

The formation of various by-products in the preparation of tetrahydrocarbazoles is discussed. An alternative mechanism for this reaction (Pausacker and Schubert, *Nature*, in the press) is considered.

Although many mechanisms * have been advanced for the Fischer indole synthesis (cf. Hollins, "The Chemistry of Nitrogen Ring Compounds," Benn Bros., pp. 92 *et seq.*), Robinson and Robinson (*J.*, 1918, **113**, 639; 1924, **127**, 827) have propounded the most satisfactory mechanism for this reaction. A slight modification has been introduced by Allen and Wilson (*J. Amer. Chem. Soc.*, 1943, **65**, 611).

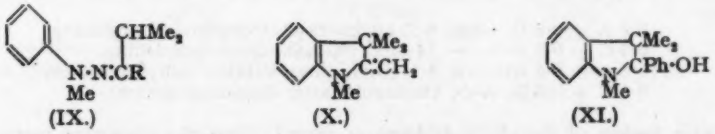
* Carlin and Fisher (*J. Amer. Chem. Soc.*, 1948, **70**, 3421) have recently proposed an entirely new mechanism for this reaction. Their results will not be discussed in the present communication.

For the cyclisation of *cyclo*hexanone phenylhydrazone (I), the modified mechanism would be represented as follows :



Additional evidence may now be cited in support of several of these stages. Grammaticakis (*Bull. Soc. chim.*, 1947, **14**, 438) has recently shown that two forms of the phenylhydrazone of *cyclo*hexanone (I and II) can be proved by spectrographic methods to coexist in equilibrium. Furthermore, the conversion of (IV) (in its hydrolysed keto-form) into (VI) is rendered probable by Reissert and Heller's observation (*Ber.*, 1904, **37**, 4369, 4375) that (VII) (formed from 1-chloro-2 : 4-dinitrobenzene and ethyl acetoacetate) is converted into 6-amino-3-carbethoxy-2-methylindole (VIII) in the presence of tin, stannous chloride, and hydrochloric acid.

The possibility of hydrolysis of the intermediate imine (IV) is also supported by the observed course of cyclisation of phenylhydrazones of type (IX), the product being (X) or (XI) according as R = CH_3 or Ph (Plancher, *Ber.*, 1898, **31**, 1496; Jennisch, *Monatsh.*, 1906, **27**, 1223).

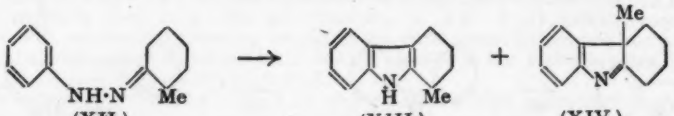


When the above evidence is combined with that already cited by Robinson and Robinson (*loc. cit.*) it is seen that only stage (*b*), involving an ortho-benzidine type rearrangement, is unsupported by very strong, experimental evidence, although even here certain analogies have been obtained. Despite a contrary statement by Robinson and Robinson, it is felt that an appreciable amount of para-rearrangement should occur concurrently, leading, as already stated by the above authors, to the formation of *p*-aminophenylacetaldehydes, which would be expected to self-condense. It may be noted that tetrahydrocarbazole can be prepared in yields exceeding 90%, indicating that here at least, para-rearrangement is inappreciable. Furthermore, the halogen atom in *o*-substituted phenylhydrazones of *cyclo*hexanone appears to be labile under the gentle conditions of cyclisation, forming hydroxytetrahydro*iso*carbazoles (see Part I, preceding paper), and this activation of halogen cannot be reconciled with the accepted interpretation of the benzidine rearrangement. However, it may be argued that the tetrahydro*iso*carbazoles are formed by an entirely different mechanism.

When an attempt was made to distinguish between an inter- and an intra-molecular mechanism for this reaction, initial results indicated that it was intermolecular in nature, thus differing from the benzidine rearrangement. In the evidence to be cited, it may be noted that the substituted phenylhydrazones of different *cyclo*hexanones were used owing to their ease of cyclisation.

After cyclising a mixture of the *p*-tolylhydrazone of *cyclo*hexanone and the phenylhydrazone of 4-methyl*cyclo*hexanone with dilute sulphuric acid, the tetrahydrocarbazole fraction was dehydrogenated with palladised charcoal, and a mixture of carbazoles was obtained. 3-Methyl- and 3 : 6-dimethyl-carbazole were separated from the mixture by fractional crystallisation and carbazole was detected by infra-red analysis. If the reaction were truly intramolecular, then

the two initial products, *i.e.*, 3-methyl- and 6-methyl-1 : 2 : 3 : 4-tetrahydrocarbazole would give the same product, *viz.*, 3-methylcarbazole, upon dehydrogenation. Further evidence was obtained from a study of the cyclisation (glacial acetic acid) of a mixture of equal weights of the *o*-tolylhydrazone of *cyclo*hexanone and the phenylhydrazone of 2-methyl*cyclo*hexanone (XII). An intramolecular reaction would lead to formation of 8-methyl- and 1-methyl-1 : 2 : 3 : 4-tetrahydrocarbazole (XIII) and the base 11-methyl-1 : 2 : 3 : 4-tetrahydrocarbazolenine (XIV) [cf. Plancher, *Atti R. Accad. Lincei*, 1900, (i), **9**, 221; Beilstein, Vol. 20, 420]:



(XII.) (XIII.) (XIV.)

On the other hand, intermolecular reaction could lead to the formation of 1 : 2 : 3 : 4-tetrahydro carbazole and its 1 : 8-*dimethyl* derivative and 8 : 11-dimethyl-1 : 2 : 3 : 4-tetrahydrocarbazolenine (XV), as well as the three products mentioned above. It was found that approximately equal weights of the basic and neutral fractions were obtained in good yield, the basic fraction consisting of an approximately equimolar mixture of (XIV) and the 8 : 11-dimethyl compound (XV). This was proved by thermal analysis of its picrate, which was also separated into the two components by fractional crystallisation. The neutral product, after dehydrogenation, yielded carbazole and 1-methylcarbazole only. Once again an intermolecular reaction was indicated.

The assumption was therefore made that fission of the N–N linkage had occurred, and the fragments formed were capable of recombination leading to indole formation. For example, the last cyclisation may be schematically represented as follows:

(A) (B) (C) (D)

0·5 A + 0·5 D ⟶ 8 : 11-Dimethyltetrahydrocarbazolenine.
0·5 C + 0·5 D ⟶ 11-Methyltetrahydrocarbazolenine.
0·5 A + 0·5 B ⟶ 1-Methylcarbazole (after dehydrogenation).
0·5 C + 0·5 B ⟶ Carbazole (after dehydrogenation).

If hydrolytic fission of the N–N linkage occurred, then the products from *cyclo*hexanone phenylhydrazone would be either aniline and *cyclo*hexanone oxime (XVI) or phenylhydroxylamine and *cyclo*hexaneimine (which would be further hydrolysed to *cyclo*hexanone). After heating a solution of aniline and (XVI) in glacial acetic acid under reflux, only unchanged (XVI) and acetanilide could be recovered. This agrees with Robinson and Robinson's observation (*loc. cit.*) that added amines did not enter into the reaction. Similarly, tetrahydrocarbazole could not be isolated after phenylhydroxylamine and *cyclo*hexanone had been heated in glacial acetic acid solution.

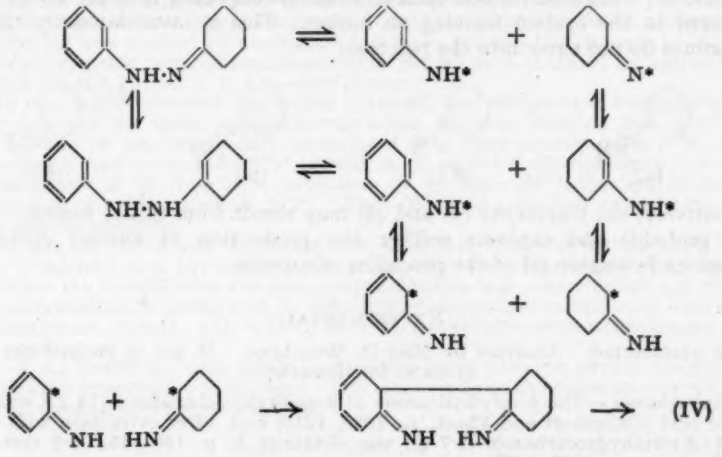
The most plausible alternative to the above involves a homolytic fission of the N–N bond, forming free radicals. The mechanism initially given would then be modified as set out at the top of the opposite page. Stages (*c*), (*d*), and (*e*) would remain unaltered.

Sir Robert Robinson (private communication) has indicated that the results obtained in the mixed cyclisation may be simply interpreted by assuming a simple hydrolysis of the hydrazone followed by condensation as shown below:

$$\left.\begin{matrix}\text{A·NH·N:B}\\ \text{C·NH·N:D}\end{matrix}\right\} \longrightarrow \left.\begin{matrix}\text{A·NH·NH}_2 + \text{O:B}\\ \text{C·NH·NH}_2 + \text{O:D}\end{matrix}\right\} \longrightarrow \left.\begin{matrix}\text{A·NH·N:B + A·NH·N:D}\\ \text{+ C·NH·N:B + C·NH·N:D}\end{matrix}\right.$$

The four phenylhydrazones thus obtained could then be cyclised each to its own indole. This hypothesis could not be tested fully but it was found at least that when the *o*-tolylhydrazone of *cyclo*hexanone was cyclised (glacial acetic acid) in the presence of phenylhydrazine, carbazole could be isolated in appreciable quantity from the dehydrogenated product. This indicates that the above suggestion is of vital importance, for it provides a simpler alternative for the free-radical mechanism. However, it is considered that tetrahydro*iso*carbazole formation, from mono- and di-*o*-substituted phenylhydrazones, takes place by the free-radical mechanism.

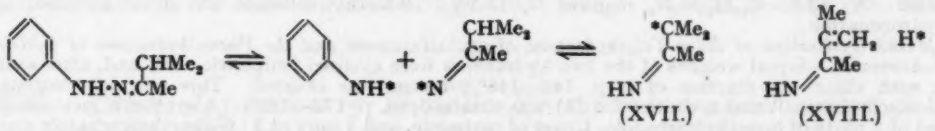
It is intended to examine the kinetics of the Fischer indole synthesis in the hope of obtaining a final decision between the two alternatives given above for stage (*b*).



It may be noted that the following examples are in harmony with the free-radical mechanism :

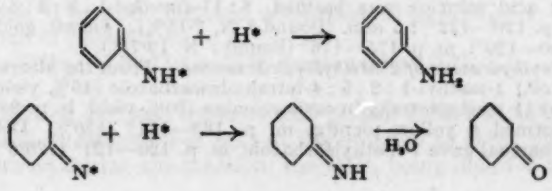
(*a*) The hydrolysis of the *o*-halogen in a substituted phenylhydrazone and the formation of tetrahydro*iso*carbazoles can be readily explained. This statement depends on the validity of the structure assigned to these compounds, which have not yet been synthesised by an unambiguous method.

(*b*) The facile cyclisation of methyl *iso*propyl ketone phenylhydrazone (Plancher, *loc. cit.*) may be explained, as the intermediate free radical (XVII) formed would be stabilised by hyperconjugative contributions of type (XVIII). In addition it may be noted that the frag-



ment D (p. 1386) should be similarly stabilised as (XIX) and not as (XX). This hypothesis is justified as it is found (*loc. cit.*) that D enters into tetrahydrocarbazolenine formation only.

(*c*) As already mentioned (see Part I), the corresponding anilines were produced in small quantities during the cyclisation of the *o*-anisyl- and *o*-chlorophenyl-hydrazones of *cyclo*hexanone. In addition, it has been found that aniline and *o*-toluidine (identified as their acetyl derivatives) were formed in the cyclisation of the phenyl- and *o*-tolyl-hydrazones of *cyclo*hexanone. Furthermore, the probable formation of small amounts of *cyclo*hexanone is also indicated, by both its odour and boiling point, in all these cyclisations. In agreement with the known properties of free radicals, the formation of these products is explained by assuming hydrogen radical abstraction from some part of the system, *i.e.* :



Of course, if the N–N bond does break, then heterolytic fission must also be considered, although it is not favoured at present for the following reasons:

(i) If (α) and (β) were first formed then it would be expected that (α) would combine with a proton present in the system forming an amine. This is invalidated by the observation that added amines do not enter into the reaction.

(α.) (β.) (γ.) (δ.)

(ii) Alternatively, the fragments (γ) and (δ) may result from initial fission. This mode of break is less probable and explains neither the production of amines during cyclisation nor the facts given in section (*b*) of the preceding discussion.

Experimental.

(All m. p.s are uncorrected. Analyses by Miss D. Wauchope. M. p.s in parentheses refer to those given in the literature.)

3 : 6-*Dimethylcarbazole*.—The *p*-tolylhydrazone of 4-methyl*cyclo*hexanone (14 g.) was cyclised with dilute sulphuric acid (Oakeshott and Plant, *J.*, 1926, 1210) and, after extraction with ether, 3 : 6-dimethyl-1 : 2 : 3 : 4-tetrahydrocarbazole (8·7 g.) was obtained, b. p. 150—152°/0·5 mm., m. p. 106—108° (112°); picrate, m. p. 146° (147°).

The tetrahydrocarbazole was dehydrogenated (6 hrs. at 280—300°) with palladised charcoal in a stream of hydrogen, yielding 3 : 6-dimethylcarbazole, which crystallised from light petroleum (b. p. 60—90°) as small white needles, m. p. 217—218° (219°); picrate, m. p. 192° (192°).

3-*Methylcarbazole*.—3-Methyl-1 : 2 : 3 : 4-tetrahydrocarbazole was prepared according to Plant and Rosser's method (*J.*, 1928, 2454), m. p. 108° (109—110°); *picrate*, copper flakes crystallising from benzene–light petroleum (b. p. 60—90°), m. p. 134—135° (Found: N, 13·0. $C_{18}H_{15}O_7N_4$ requires N, 13·5%). Dehydrogenation with palladised charcoal gave 3-methylcarbazole, m. p. 204° (203°); picrate, m. p. 182—183° (180°).

Similarly, the cyclisation of the *p*-tolylhydrazone of *cyclo*hexanone (Borsche, Witte, and Bothe, *Annalen*, 1908, **359**, 49) yielded 6-methyl-1 : 2 : 3 : 4-tetrahydrocarbazole, b. p. 160°/0·2 mm., m. p. 138° (141—142°); *picrate*, dark brown needles from benzene–light petroleum (b. p. 60—90°), m. p. 149° (Found: N, 13·2. $C_{19}H_{18}O_7N_4$ requires N, 13·5%). 3-Methylcarbazole was again obtained after dehydrogenation.

Mixed Cyclisation of the p-*Tolylhydrazone of* cyclo*Hexanone and the Phenylhydrazone of* 4-*Methyl*cyclo*hexanone*.—Equal weights of the two hydrazones were cyclised (sulphuric acid) and, after extraction with ether, the fraction of b. p. 140—148°/0·4 mm. was isolated. This was dehydrogenated (palladised charcoal) and a white solid (A) was obtained, m. p. 172—188°. A synthetic mixture composed of 2 parts of 3-methylcarbazole, 1 part of carbazole, and 1 part of 3 : 6-dimethylcarbazole melted at 173—187°, and when mixed with (A) melted at 174—187°.

After fractional crystallisation of the picrate of (A), almost pure 3 : 6-dimethylcarbazole picrate (m. p. 186—187°) was obtained which had the following m. p.s: mixed with carbazole picrate (m. p. 186°), 165—168°; mixed with 3-methylcarbazole picrate (m. p. 182—183°), 180—181°; mixed with 3 : 6-dimethylcarbazole picrate (m. p. 192°), 188—190°; mixed with last picrate and carbazole picrate, 181—182°.

The more volatile portion from the fractional vacuum sublimation of (A) yielded 3-methylcarbazole (m. p. 198—200°) after crystallisation from benzene. Mixed m. p. with an authentic specimen, 199—201°.

Thus evidence of the presence of both 3-methyl- and 3 : 6-dimethyl-carbazole in (A) has been obtained. Furthermore, Mr. A. Walsh of the Division of Industrial Chemistry, Council for Scientific and Industrial Research, has detected the presence of carbazole in (A) by infra-red spectroscopic analysis.

Cyclisation of the o-*Tolylhydrazone of* 2-*Methyl*cyclo*hexanone*.—When the above cyclisation was performed (sulphuric acid), 1 : 8-*dimethyl*-1 : 2 : 3 : 4-*tetrahydrocarbazole* (3·3 g., b. p. 145—148°/1 mm.) was isolated from the ethereal extract (Found: N, 6·9. $C_{14}H_{17}N$ requires N, 7·0%); *picrate*, dark red needles from light petroleum (b. p. 100—120°), m. p. 135—136° (Found: N, 13·6. $C_{20}H_{20}O_7N_4$ requires N, 13·1%). After dehydrogenation of this tetrahydrocarbazole with palladised charcoal, 1 : 8-*dimethylcarbazole* was obtained, crystallising from light petroleum (b. p. 60—90°) as white needles, m. p. 175—176° (Found: N, 7·2. $C_{14}H_{13}N$ requires N, 7·2%); *picrate*, orange needles from light petroleum (b. p. 60—90°), m. p. 174° (Found: N, 13·8. $C_{20}H_{16}O_7N_4$ requires N, 13·2%).

When the sulphuric acid solution was basified, 8 : 11-*dimethyl*-1 : 2 : 3 : 4-*tetrahydrocarbazolenine* (1·9 g.) was isolated, b. p. 120—122°/1·5 mm. (Found: N, 7·15%); *picrate*, golden-yellow plates from light petroleum (b. p. 100—120°), m. p. 175—176° (Found: N, 12·7%).

Cyclisation of the Phenylhydrazone of 2-*Methyl*cyclo*hexanone*.—From the above cyclisation (sulphuric acid) (cf. Plancher, *loc. cit.*) 1-methyl-1 : 2 : 3 : 4-tetrahydrocarbazole [45% yield, b. p. 142—146°/0·3 mm., m. p. 69° (72°)] and 11-methyltetrahydrocarbazolenine (20% yield, b. p. 98—100°/0·3 mm.) were obtained. The latter formed a yellow picrate, m. p. 169—171° (170°). Dehydrogenation of the former with palladised charcoal gave 1-methylcarbazole, m. p. 120—121° (120·5°); picrate, m. p. 143° (143·5°).

Mixed Cyclisation of the o-*Tolylhydrazone of* cyclo*Hexanone and the Phenylhydrazone of* 2-*Methyl*cyclo*hexanone.*—A mixture (14·6 g.) of equal weights of the above two compounds was added to boiling glacial acetic acid (200 ml.). After the initial vigorous reaction, the solution was heated under reflux (15 minutes), and the acetic acid removed under reduced pressure (water-bath). Dilute hydrochloric acid was added, and the crude tetrahydrocarbazoles (7·0 g.) extracted with ether. The pure tetrahydrocarbazole fraction (B) was isolated by distillation (5·0 g., b. p. 150—152°/1·5 mm.). After basification of the acidic solution, crude tetrahydrocarbazolenines (6·2 g.) were obtained by extraction with ether. The pure fraction (C) (4·6 g.) had b. p. 120—122°/2 mm.

Fraction (B) was dehydrogenated (palladised charcoal), and the product fractionally sublimed in a vacuum. The m. p.s of the three, approximately equal, fractions were (*a*) 105—150°, (*b*) 125—195°, (*c*) 220—223°. When (*a*) was fractionally crystallised from light petroleum (b. p. 60—90°), the more soluble portion finally had m. p. 119—120° (mixed m. p. with 1-methylcarbazole, 120—121°). After repeated crystallisation of (*c*) from light petroleum (b. p. 100—120°), the less soluble fraction had m. p. 242° (mixed m. p. with carbazole, 242°). Thus (B) gives a mixture of carbazole and 1-methylcarbazole after dehydrogenation.

Fraction (C) was converted into its picrate and, after four recrystallisations from light petroleum (b. p. 100—120°), this had m. p. 167—170° (mixed m. p. with 11-methyltetrahydrocarbazolenine picrate, 168—171°). When the filtrate from the first recrystallisation was concentrated and the crystals thus obtained were recrystallised, a product, m. p. 166—168°, was obtained (mixed m. p. with 8 : 11-dimethyltetrahydrocarbazolenine picrate, 171—172°; with 11-methyltetrahydrocarbazolenine picrate, 143—159°).

Treatment of (C) (0·095 g.) with picric acid (0·109 g.) in benzene gave a picrate (shrinks 133°, m. p. 143—154°) after evaporation to dryness. This is shown to be an approximately equimolar mixture of the above two picrates by thermal analysis.

	(i.)	(ii.)	(iii.)	(iv.)	(v.)	(vi.)	(vii.)
11-Methyltetrahydrocarbazolenine picrate, %	22	34	48	56	68	79	93
Shrinking pt.	130°	127°	115°	115°	138°	148°	166°
M. p.	161—165°	149—159°	145—156°	148—158°	152—156°	159—163°	167—185°

Mixed m. p. of (iii) with the picrate of (C), 144—155° (shrinks 125°).

Cyclisation of the o-*Tolylhydrazone of* cyclo*Hexanone in the Presence of Phenylhydrazine.*—The hydrazone was added to an equimolar amount of phenylhydrazine in boiling glacial acetic acid. The tetrahydrocarbazole fraction was dehydrogenated, and the product fractionally sublimed in a vacuum (see above). The last fraction, when crystallised from benzene, had m. p. 242° (mixed m. p. with carbazole, 242°).

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296. *The Halogenation of Phenolic Ethers and Anilides. Part XV. The Kinetics of Bromination in 75% Acetic Acid.*

By A. E. BRADFIELD, G. I. DAVIES, and (MISS) E. LONG.

The rate of bromination of *p*-chlorophenyl ethers in 75% acetic acid by bromine solutions is represented by the expression: $dx/dt = k_2[E][Br_2] + k_3[E][Br_2]^2$, the ratio k_3/k_2 being constant. The same expression is applicable to *o*-chlorophenyl ethers, but the ratio k_3/k_2 is approximately one fifth of that found for the *p*-chloro-series. Hypobromous acid is a powerful brominating agent for ethers in this medium, but analysis of the kinetics of the reaction shows that it plays little, if any, part in bromination by bromine solutions. The significance of the term involving $[Br_2]^2$ is discussed.

(*Symbols.*—The following symbols are used throughout with the meaning shown, concentrations being in g.-mols./l.$^{-1}$:

a = total initial concentration of bromine.
b = total initial concentration of hydrobromic acid.
c = initial concentration of phenol ether.
$[Br_2]$ = concentration of bromine not combined as HBr_3
$= \frac{1}{2}\{\sqrt{(K + b - a + 2x)^2 + 4K(a - x)} - (K + b - a + 2x)\} = y.$
$[HBr]$ = concentration of hydrobromic acid not combined as $HBr_3 = b + x - (a - x - y)$.
$[E]$ = concentration of phenolic ether.
$K = [Br_2][HBr]/[HBr_3] = 0{\cdot}0110$ in 75% acetic acid at 20°).

KINETICALLY, the reaction between bromine and phenol ethers in 50% acetic acid is, in the first place, complicated by retention of part of the bromine as HBr_3 by the hydrobromic acid added initially or formed during the reaction, the HBr_3 being inactive as a brominating agent

in this medium. It was found by Bradfield, Jones, and Orton (*J.*, 1929, 2810) that, provided that 5—10 molecular proportions of hydrobromic acid were present throughout the reaction, the rate of reaction could be represented reasonably well as a bimolecular reaction between "free" bromine and ether:

$$dx/dt = k_b[E][Br_2] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The bimolecular constant k_b tends to fall somewhat as the reaction progresses, this fall being less marked when ten than when five molecular proportions of bromine are present initially. An important observation of these authors was that, when two different ethers of the *p*-chloro- or *p*-bromo-series are brominated under identical conditions, and the times t_1 and t_2, respectively, taken for a given percentage change compared, the ratio $t_1 : t_2$ is constant at all stages of the reaction, *i.e.*, the relative rates of bromination are equal to the inverse ratio of the times taken for a given percentage change. From this it was inferred that, for the ethers examined, the law governing the rate of reaction may be expressed by an equation containing only one constant characteristic of the ether employed, *i.e.*, of the form:

$$dx/dt = k\phi([E], [Br_2], [HBr]) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

notwithstanding the possible existence of complex equilibria between the various molecular species which may be present.

There is good reason to believe that, with bromine solutions, the bromine molecule is not the only brominating agent, particularly in aqueous media. Thus, to account for the large accelerating influence of water on the bromination of phenol in carbon tetrachloride, Baines (*J.*, 1922, 2810) assumed that the bromine molecule is but a weak brominating agent, while hypobromous acid, formed by hydrolysis, is very powerful. On the other hand, Francis (*J. Amer. Chem. Soc.*, 1925, **47**, 2340) found that *m*-nitrophenol is brominated about 1000 as fast by bromine-water as by aqueous hypobromous acid and ascribed the activity of bromine-water to bromine atoms, probably carrying positive charges. A somewhat similar view is held by Schilov and Naniaev (*Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **24**, 890), who found that the rate of bromination of anisole-*m*-sulphonic acid in water by hypobromous acid can be represented by the equation: $-d[HOBr]/dt = k[A][HOBr][H^+]$, and suggested that $[HOBr][H^+]$ is a measure of the formation of the bromine cation: $HOBr + H^+ \longrightarrow Br^+ + H_2O$, as a very powerful brominating agent. On the other hand, Alexander (*J.*, 1938, 729), overcoming the difficulty presented by the extreme rapidity of bromination of phenols in aqueous solution by spreading *p*-hexadecylphenol as a monolayer on an aqueous substrate, found that in bromine-water the effective brominating agents are the tribromide ion and the hypobromous acid molecule, the former being four times as active as the latter. Israel, Tuck, and Soper (*J.*, 1945, 547) showed that in chloroform solution a brominating agent is produced, from a bromo-amine and acetic acid, which they consider to be the acyl hypobromite and which is capable of brominating anisole and phenetole.

In acetic acid, Robertson and his collaborators (*J.*, 1943, 276; 1948, 100) found that the order of reaction between bromine and a variety of substances varies between two and four as the concentration of the bromine is increased, and put forward the expression:

$$dx/dt = k[A][Br_2] + k'[A][Br_2]^3 + k''[A][Br_2]^4 \quad . \quad . \quad . \quad . \quad . \quad (3)$$

As their measurements were generally confined to the early stages of the reaction, the formation of HBr_3 was neglected, and the bromine concentrations terms represent the total bromine concentration. No attempt was made to apply this equation in detail. The interpretation placed on the term involving the square of the bromine concentration is considered later.

The present investigation is concerned primarily with an attempt to elucidate further the kinetics of bromination by bromine in 75% acetic acid; velocity measurements have been made with *o*- and *p*-chlorophenyl ethers, the initial conditions being varied. Some experiments on bromination by hypobromous acid in the same medium have also been made.

Experimental.

Perbromide Equilibrium Constant.—The perbromide equilibrium constant in 75% acetic acid was determined by the aspiration method of W. J. Jones (*J.*, 1911, **99**, 392), using an apparatus in which all connections in contact with bromine vapour were of ground glass, lubricated with phosphoric acid. For the solubility coefficient of bromine at 20°, a value of 260 was found. The following table gives illustrative figures of the equilibrium-constant determinations at 20°, for which a mean value of $K = 0{\cdot}0110$ was found.

$a - b$ = c.c. of 0·01734N-thiosulphate equivalent to bromine carried over by V c.c. of air.

	Initial concentrations. g.-mols./l.				
V.	Total Br.	Total HBr.	$a - b$.	Concn. of free Br.	K.
1038	0·0375	0·0188	11·49	0·0246	0·0111
996	0·0371	0·0188	10·81	0·0241	0·0107
873	0·0283	0·00953	8·68	0·0220	0·0114
1127	0·0189	0·01875	5·05	0·00991	0·0107

Determination of Velocity of Bromination by Bromine.—Materials were prepared or purified and velocity determinations carried out by the method of Bradfield, Jones, and Orton (*J.*, 1929, 2810). The solvent used consisted of a mixture of purified acetic acid and water in the proportion of 3 : 1 by weight. Two series of measurements were made of the rate of bromination at 20° of the ethers of *o*- and *p*-chlorophenol, respectively, with the initial concentration of hydrobromic acid varied from ten times the bromine concentration to zero. The data obtained were plotted as x–t curves, and with each series of ethers, relative rates were obtained directly from the curves representing experiments in which the initial conditions were identical. The same constancy of the ratio $t_1 : t_2$ is observed when $b = 0$ as when $b = 5a$ or $10a$.

The necessity of allowing for HBr_3 formation leads to a somewhat unwieldy expression when the differential equation corresponding to a bimolecular reaction between bromine and ether is integrated. To simplify the task of testing the alternative hypotheses concerning the reaction mechanism, it was therefore decided to make direct use of values of dx/dt obtained from the x–t curves. To overcome the well-known difficulty of drawing accurate tangents to a curve of large radius of curvature, the device of Groocock, Ingold, and Jackson (*J.*, 1930, 1039) was at first employed by us, but later more accurate values for the tangents were calculated in the following manner It was found that the curves representing the disappearance of bromine could be accurately reproduced by empirical equations of the following form : $t = \alpha n + \beta n^2 + \gamma n^3 + \delta n^4$, where n is the fraction of the bromine which has disappeared in t minutes. In each case, from a smooth curve drawn through the experimental points, values of t corresponding to $n = 0{\cdot}1$, 0·2, etc., were read off, and from these figures the constants α, β, γ, and δ were evaluated. Since $na = x$, $\frac{dn}{dx} = \frac{1}{a}$ and $\frac{dt}{dx} = \frac{dn}{dx} \cdot \frac{dt}{dn} = \frac{1}{a} \cdot \frac{dt}{dn}$, while $\frac{dt}{dn} = \alpha + 2\beta n + 3\gamma n^2 + 4\delta n^3$, numerical values of dt/dx and hence of dx/dt are easily calculated. Substitution of numerical values of dx/dt and of the concentration terms in equation (1) and the other differential equations considered below has been used throughout this paper as a substitute for integration for the evaluation of the constants k_b, etc.

As examples of the experimental figures, data are given in Table I for the bromination of *p*-chlorophenyl *iso*propyl ether at 20°.

TABLE I.

(Concentrations in g.-mols./l., t = time in minutes, $S = dx/dt \times 10^6$.)

Exps. I—IV $\begin{cases} a = 0{\cdot}0075 \\ c = 0{\cdot}0375 \end{cases}$ Exp. V $\begin{cases} a = 0{\cdot}01875 \\ c = 0{\cdot}01875 \end{cases}$ Exp. VI $\begin{cases} a = 0{\cdot}0375 \\ c = 0{\cdot}01875 \end{cases}$

Exp.	% change :	30	35	40	45	50	55	60
I ($= 10a$)	t =	378	463	556	666	792	938	1102
	S =	4·81	4·20	3·65	3·17	2·76	2·41	2·12
II ($b = 5a$)	t =	214	265	325	392	467	549	651
	S =	7·68	6·76	5·93	5·22	4·60	4·06	3·61
III ($b = 3a$)	t =	136	170	209	253	307	367	437
	S =	11·9	10·4	9·01	7·76	6·66	5·69	4·85
IV ($b = a$)	t =	63	80·5	99	122	151	185	226
	S =	24·4	20·9	17·7	14·8	12·3	10·3	8·53
V ($b = 0$)	t =	41	58	79	108	148	210	298
	S =	72·5	52·4	36·6	25·4	17·9	12·9	9·46
VI ($b = 0$)	t =	8·2	10·6	13·6	17·2	21·7	27·7	35
	S =	438	350	290	229	180	142	112

The rate is practically the same when five molecular proportions of sodium bromide are added initially as when the equivalent amount of hydrobromic acid is used. Again, in the absence of added hydrobromic acid, the presence of three molecular proportions of β-naphthalenesulphonic acid is without appreciable effect. It would be expected, of course, that the buffering effect of the 75% acetic acid would largely mask any possible consequence of small variations of strong-acid content on the rate of bromination.

Bromination by Hypobromous Acid.—A solution of hypobromous acid was prepared by shaking bromine, water, and yellow mercuric oxide. Acetic acid was added to this solution to bring the composition to 75% acetic acid. To estimate the small amount of bromine sometimes present, use was made of the fact that *p*-chlorophenyl 4-nitrobenzyl ether reacts almost instantaneously with hypobromous acid in 75% acetic acid and is scarcely attacked by bromine. A measured quantity of the hypobromous acid solution was added to an excess of this ether, and the remaining bromine could then be titrated. Allowance for the bromine present was made when necessary.

The hypobromous acid solution reacted almost instantaneously with equimolecular accounts of *o*- or *p*-chloroanisole or similar ethers. In some cases the product was isolated. For example, *p*-chlororophenyl 4-nitrobenzyl ether (1·253 g.) in 75% acetic acid was mixed with 0·05M-hypobromous

acid (100 c.c.) and made up to 500 c.c. When the mixture was shaken, the product crystallised. It had m. p. 142—143°, and, after crystallisation from alcohol, m. p. 146—147° (0·1279 G. of the crude product gave 0·1211 g. of mixed silver halides. Calc. for $C_{13}H_9NO_3ClBr$: 0·1237 g. 0·1107 G. of the recrystallised substance gave 0·1068 g. of silver halides. Calc.: 0·1070 g.). Similarly from *p*-nitrophenetole, 2-bromo-4-nitrophenetole, m. p. 97—98°, was obtained.

The rate of bromination by hypobromous acid was, in most cases, much too rapid to be measured. Approximate values, calculated for a bimolecular reaction, were, however, obtained for *p*-nitrophenetole and *p*-nitrophenyl benzyl ether, as shown below:

[HOBr] = [Ether] = 0·008 g. mols./l.

p-Nitrophenetole.			*p*-Nitrophenyl benzyl ether.		
t.	% change.	*k*.	*t*.	% change.	*k*.
7·4	62·4	18·8	13·3	54·0	8·19
11·4	74·8	17·8	24·5	73·9	8·17

Discussion.

In Table II is shown the effect of varying the molecular proportion of hydrobromic acid initially present on the bimolecular coefficient k_b for the bromination of *p*-chlorophenyl *iso*propyl ether, calculated from equation (1) by the method described above. It will be seen that, when $b = 0$, k_b is not only far from constant, but numerically from about two to six times greater than when $b = 10a$. On the other hand, a moderately good termolecular constant, k_t,

$$\frac{dx}{dt} = k_t[E][Br_2]^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

derived from equation (4) may be calculated for Experiment VI.

In view of these results, the possibility of the simultaneous occurrence of a bimolecular and a termolecular reaction, as represented by equation (5) was examined. Suitable pairs of equations

$$\frac{dx}{dt} = k_b[E][Br_2] + k_t[E][Br_2]^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

were solved for k_t/k_b. For *p*-chlorophenyl *iso*propyl ether, a mean value of $k_t/k_b = 319$ was adopted and k_b calculated, with the results shown in Table III. In Table IV are given the values of k_b for the methyl, ethyl, and *n*-propyl ethers of *p*-chlorophenol, using the same fixed value of $k_t/k_b = 319$.

Because of the retention of part of the bromine in an inactive form as HBr_3, the experiments quoted in Table III represent a more considerable range than is at first apparent. The initial "free" bromine concentration increases from 0·00103 g.-mol./l. (13·8% of the total bromine) in Exp. I to 0·0375 g.-mol./l. in Exp. VI. Correspondingly the initial values of the ratio

Table II.

Values of k_b *and* k_t, *calculated by equations* (1) *and* (4) *for the bromination of* p-*chlorophenyl* iso*propyl ether in* 75% *acetic acid.*

Exp.	% change:	30	35	40	45	50	55	60
I ($b = 10a$)	k_b =	0·199	0·191	0·183	0·176	0·172	0·170	0·172
IV ($b = 5a$)	k_b =	0·222	0·210	0·200	0·190	0·180	0·173	0·167
V $\{b = 0, a = c\}$	k_b =	0·531	0·468	0·403	0·352	0·316	0·296	0·291
VI $\{b = 0, a = 2c\}$	k_b =	1·22	1·14	1·06	0·97	0·90	0·84	0·80
	k_t =	43·1	42·7	41·8	40·8	40·1	40·0	40·8

Table III.

Bromination of p-*chlorophenyl* iso*propyl ether.*
Values of k_b *calculated by equation* (5), *with* $k_t/k_b = 319$.

Exp.	% change:	30	35	40	45	50	55	60	Mean.
I ($b = 10a$)		0·163	0·159	0·154	0·151	0·150	0·150	0·154	0·154
II ($b = 5a$)		0·128	0·127	0·127	0·127	0·130	0·134	0·140	0·130
III ($b = 3a$)		0·119	0·119	0·119	0·119	0·120	0·121	0·123	0·120
IV ($b = a$)		0·111	0·110	0·110	0·110	0·109	0·110	0·112	0·110
V $\{b = 0, a = c\}$		0·123	0·119	0·113	0·109	0·108	0·112	0·122	0·115
VI $\{b = 0, a = 2c\}$		0·122	0·120	0·116	0·113	0·110	0·109	0·110	0·114

TABLE IV.

Values of k_b *calculated by equation* (5) *with* $k_1/k_b = 319$.

Exp. VII. *p*-Chloroanisole
Exp. VIII. *p*-Chlorophenetole
Exp. IX. *p*-Chlorophenyl *n*-propyl ether
} Concns. as in Exp. V.

Exp.	% change : 30	35	40	45	50	55	60	Mean.
VII.	0·0141	0·0138	0·0136	0·0135	0·0133	0·0135	0·0140	0·0137
VIII.	0·0375	0·0365	0·0351	0·0337	0·0325	0·0319	0·0320	0·0341
IX.	0·0485	0·0467	0·0451	0·0438	0·0431	0·0433	0·0426	0·0447

$[E]/[Br_2]$ vary from 36·4 : 1 to 0·5 : 1. In the slowest reaction (experiment I), 792 minutes are required for disappearance of 50% of the bromine, with $dx/dt = 2·77 \times 10^{-4}$ at this point; the corresponding figures for the most rapid (experiment VI) are 21·75 minutes and $dx/dt = 180 \times 10^{-4}$. Bearing in mind that, because of the smoothing off of experimental errors effected by the method of calculating dx/dt, these errors do not appear as random errors in the individual values of k_b, but as a trend up or down in a given experiment, the applicability of equation (5) over this wide range of speeds is very satisfactory. Quite distinct from this trend in some experiments, there is a steady change in the mean value of k_b from 0·154 in experiment I ($b = 10a$) to about 0·113 in experiments IV, V, and VI ($b = a$ or 0). This change is similar to that shown by the bimolecular velocity coefficients for the chlorination of *p*-chloroanisole in 99% acetic acid, Bradfield and Jones (*J.*, 1928, 1006) finding $k = 1·41$, 1·32, and 1·22 when 7·0, 4·0, and 0·2 molecular proportions, respectively, of hydrochloric acid over and above that required to liberate chlorine from the chloro-amine were present in the reaction mixture.

A more limited number of measurements have been made with *o*-chlorophenyl ethers, for which the rate of bromination is intrinsically greater than for the corresponding *p*-chlorophenyl ethers. It is found that, in the *o*-chloro-series, k_1/k_b is 63·5, *i.e.*, about 1/5th of its value in the *p*-chloro-series. Calculated values of k_b for *o*-chloroanisole are given in Table V, showing that in this case also equation (5) is applicable, though with this ether the direction of change in the mean value of k_b with changing concentration of hydrobromic acid is the reverse of that observed with *p*-chlorophenyl *iso*propyl ether.

TABLE V.

Bromination of o-chloroanisole.

Values of k_b *calculated by equation* (5) *with* $k_1/k_b = 63·5$.

Concns. : $a = 0·0075$, $c = 0·0150$ g.-mol./l.

Exp.	% change : 30	35	40	45	50	55	60	Mean.
X ($b = 5a$)	2·81	2·75	2·71	2·67	2·65	2·65	2·69	2·70
XI ($b = a$)	3·53	3·41	3·29	3·14	3·09	3·04	3·05	3·22
XII ($b = 0$)	3·26	3·31	3·36	3·35	3·33	3·29	3·29	3·31

We consider equation (5), viewed as an empirical equation, to represent our results satisfactorily. The constancy of k_1/k_b for each series of ethers also satisfies the requirements of equation (2). Before attempting to interpret equation (5), however, alternatives must be considered and suggestions which have been put forward regarding the nature of the brominating agents responsible for the bromination of phenols must be examined.

In 75%, as in 50%, acetic acid, the very marked retarding influence of hydrobromic acid implies that HBr_2 can be, at most, only a weak brominating agent. On the other hand, we find hypobromous acid to be extremely reactive, reaction in 75% acetic acid being almost instantaneous when any of the *o*- or *p*-chloro-ethers is treated with an approximately equimolecular proportion of this reagent. Isolation of the product in several typical cases showed it to be the normal chloro-bromo-ether, identical with that obtained by the action of bromine. In an attempt to obtain measurable rates, we examined the action of hypobromous acid on *p*-nitrophenetole and *p*-nitrophenyl benzyl ether, substances which do not react appreciably with bromine in this medium. Even with these substances, bromination is extremely rapid—in the case of the former, the development of a yellow colour suggests some accompanying oxidation. Such measurements as could be made lead to approximate values of $k = 18$ and 8, respectively, for the bimolecular velocity coefficients for bromination by hypobromous acid. It is estimated that the corresponding value of k for *p*-chlorophenyl

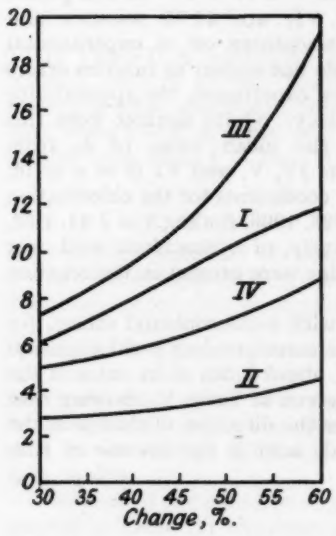
*iso*propyl ether would be 15,000—20,000. Obviously, although there is no independent evidence of bromine undergoing hydrolysis in aqueous acetic acid, the conception of bromination as due to a very low concentration of hypobromous acid provides an attractive hypothesis, and it is imperative to examine it closely.

Bromination by hypobromous acid may be represented by equations (6) and (7). Nothing

$$Br_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HOBr + HBr \quad . . . . . (6)$$

$$HOBr + EH \xrightarrow{k_2} EBr + H_2O \quad . . . . . (7)$$

is known of the relative magnitudes of k_1 and k_2, and thus three cases fall to be considered, (1) $k_1 \ll k_2$, (2) $k_1 \gg k_2$, (3) k_1 and k_2 of comparable magnitude. In each case, also, bromination must be considered as due (*a*) solely to hypobromous acid, and (*b*) partly to bromine and partly to hypobromous acid. It will be shown by reference to data from experiments V and VI above that none of these hypotheses suffices to explain the experimental results.



It is convenient first to describe the setting out of the requisite data in the figure. The initial concentration of ether, c, is the same in both experiments V and VI, but, whilst in experiment V, $a = c$, in experiment VI, $a = 2c$. Consider the experimental values of $(dx/dt)_P$ from experiment VI and $(dx/dt)_{P'}$ from experiment V for the same percentage change P. The ratio, $R = (dx/dt)_P/(dx/dt)_{P'}$ is plotted against P in curve I. In curve II are plotted the ratios of the calculated amounts of "free" bromine, *i.e.*, $[Br_2]/[Br_2]'$, $[Br_2]$ and $[Br_2]'$ referring to experiments VI and V, respectively. Similarly, in curve III the ratios, $[Br_2]^2/[Br_2]'^2$, are shown, while in curve IV are set out the ratios $([Br_2][HBr]')/([Br_2]'[HBr])$ in which [HBr] and [HBr]′ refer to values calculated for experiments VI and V, respectively.

In passing, to illustrate the use of these data, it may easily be shown that whilst the reaction is neither simply bimolecular nor termolecular, the data are compatible with equation (5) above. For, if bromination may be represented by $dx/dt = k_b[E][Br_2]$, then $R = (k_b[E][Br_2])/(k_b[E]'[Br_2]') = [Br_2]/[Br_2]'$, since $[E] = [E]'$. Curve I should then coincide with curve II. Similarly, if $dx/dt = k_t[E][Br_2]^2$, $R = [Br_2]^2/[Br_2]'^2$, and curve I should coincide with curve III. If $dx/dt = k_b[E][Br_2] + k_t[E][Br_2]^2$, then $R = (k_b[Br_2] + k_t[Br_2]^2)/(k_b[Br_2]' + k_t[Br_2]'^2)$. The numerical side of the right-hand side must lie between $[Br_2]/[Br_2]'$ and $[Br_2]^2/[Br_2]'^2$, and therefore curve I should lie between curves II and III. As it does, equation (5) is compatible with the data.

Returning now to bromination by hypobromous acid, the three cases set out above will be examined.

Case (1). Hydrolysis of bromine slow, substitution of ether very rapid, *i.e.*, $k_1 \ll k_2$.

(*a*) Bromination by HOBr only. $dx/dt = k_1[Br_2]$, $R = [Br_2]/[Br_2]'$.

(*b*) Bromination by Br_2 and HOBr.

$$dx/dt = k_b[E][Br_2] + k_1[Br_2].$$

$$R = \frac{[Br_2](k_b[E] + k_1)}{[Br_2]'(k_b[E]' + k_1)} = [Br_2]/[Br_2]' \text{ since } [E] = [E]'.$$

In both cases the values of R should coincide with curve II, a conclusion not in accord with experiment.

Case (2). Hydrolysis of bromine very fast, substitution of ether slow, *i.e.*, $k_1 \gg k_2$. In this case, [HOBr] may be taken as having its equilibrium value. It is further assumed that [HOBr] is negligible compared with $[Br_2]$, and that therefore $[Br_2]$ may be calculated in the usual way without significant error.

(*a*) Bromination by HOBr only.

$$[HOBr] = k_1[Br_2]/k_{-1}[HBr].$$

$$dx/dt = k_2[E][HOBr] = (k_1k_2[E][Br_2])/k_{-1}[HBr]; \quad R = ([Br_2][HBr]')/([Br_2]'[HBr]).$$

As the values of R do not coincide with curve IV, this is not in accord with experiment.

(*b*) Bromination by HOBr and Br_2.

$$dx/dt = k_b[E][Br_2] + k_1k_2[E][Br_2]/k_{-1}[HBr]; \; R = \frac{[E](k_b[Br_2] + k_1k_2[Br_2]/k_{-1}[HBr])}{[E]'(k_b[Br_2]' + k_1k_2[Br_2]'/k_{-1}[HBr]')}$$

The numerical value of R should lie between $[Br_2]/[Br_2]'$ and $([Br_2][HBr]')/([Br_2]'[HBr])$, *i.e.*, curve I should lie between curves II and IV. This is not in accord with experiment.

Case 3. Hydrolysis of bromine and substitution of ether proceed at comparable rates. Then, for a stationary concentration of HOBr

$$[HOBr] = k_1[Br_2]/(k_{-1}[HBr] + k_2[E]).$$

(*a*) Bromination by HOBr only.

$$dx/dt = k_2[E][HOBr] = (k_1k_2[E][Br_2])/(k_{-1}[HBr] + k_2[E]).$$

$$R = \left(\frac{[Br_2]}{k_{-1}[HBr] + k_2[E]}\right)\Big/\left(\frac{[Br_2]'}{k_{-1}[HBr]' + k_2[E]'}\right).$$

The numerical value of this expression lies between $[Br_2]/[Br_2]'$ and $([Br_2][HBr]')/([Br_2]'[HBr])$, *i.e.*, R should lie between curves II and IV. This is not in accord with experiment.

(*b*) Bromination by HOBr and Br_2.

$$dx/dt = k_b[E][Br_2] + k_1k_2[E][Br_2]/(k_{-1}[HBr] + k_2[E])$$

$$R = \frac{[E]\{k_b[Br_2] + k_1k_2[Br_2]/k_{-1}[HBr] + k_2[E])\}}{[E]'\{k_b[Br_2]' + k_1k_2[Br_2]'/(k_{-1}[HBr]' + k_2[E]')\}}$$

The numerical value of this expression lies between $[Br_2]/[Br_2]'$ and the numerical value of the expression deduced in (*a*) above, *i.e.*, R should lie between curve II and some curve which it has been deduced should lie between curves II and IV. This also is not in accord with experiment.

Notwithstanding the activity of HOBr as a brominating agent in 75% acetic acid, it thus appears that it does not participate appreciably in the bromination by bromine in this medium. The alternative formation of acetyl hypobromite in place of hypobromous acid (cf. Israel, Tuck, and Soper, *J.*, 1945, 547), $AcOH + Br_2 \rightleftharpoons AcOBr + HBr$, would not be distinguishable by our experiments. The conception of " positive bromine " as the brominating agent, in the sense used by Francis (*J. Amer. Chem. Soc.*, 1925, **47**, 2340) and Schilov and Naniaev (*Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **24**, 890), *i.e.*, a positively charged bromine atom as a separate entity, as distinct from a mesomeric polarization, may be dismissed, since the concentration of Br^+ would be proportional to $[Br_2]/[HBr]$ if bromine dissociates, or to $[HOBr][H^+]$ (Schilov and Naniaev). From what has already been said it will be clear that neither conception is compatible with the experimental results.

Returning then to the interpretation of equation (5), it seems unlikely that we are dealing with a single, unknown brominating agent X, whose concentration is a function of the " free " bromine concentration, which can be expressed, at least to a near approximation, as $[X] = \alpha[Br_2] + \beta[Br_2]^2$, for then, by hypothesis, β/α should be independent of the nature of the ether, whereas for *o*- and *p*-chloro-ethers different values of k_t/k_b are observed.

We concluded that the first term of equation (5) represents a true bimolecular reaction between the bromine molecule and the ether. As an interpretation of the second term, involving $[Br_2]^2$, either of the two mechanisms proposed by Robertson and his collaborators (*J.*, 1943, 276; 1948, 100) would suffice:

(*a*) $AH + Br_2 \rightleftharpoons AHBr_2$; $AHBr_2 + Br_2 \longrightarrow ABr + HBr + Br_2$.

(*b*) $Br_2 + Br_2 \rightleftharpoons Br_4$; $AH + Br_4 \longrightarrow ABr + HBr + Br_2$.

Of these we prefer (*b*), although no independent evidence of the existence of Br_4 molecules is available. It is implied by our calculations that the concentration of Br_4 molecules compared with Br_2 molecules is very low. The constant k_t in equation (5), on this interpretation, is really $k_b'K_B$, where k_b' is the bimolecular constant for the reaction between Br_4 and the ether, and $K_B = [Br_4]/[Br_2]^2$. It is perhaps slightly surprising to find that, for the *p*-chloro-series of ethers, k_b'/k_b is constant, as a relationship of the form, $\log k_b = m \log k_b' + constant$, might have been expected, if Br_2 and Br_4 may be regarded as two distinct reagents. It should, however, be noted that the range of velocities represented by the present series of ethers is rather small. In the case of the *o*-chloro-series, a different value of k_b'/k_b is not incompatible with this interpretation.

Finally, Table VI shows the relative effects of alkyl groups on the rate of bromination.

These values are independent of the kinetic interpretation of the measurements given above. In each series the value for the chloroanisole is taken as 100. Comparing the *o*- with the *p*-chloro-series, k_b for *o*-chloroanisole is 242 × k_b for *p*-chloroanisole. The relation of some of these relative rates of bromination to the relative rates of chlorination of the same ethers has been briefly discussed by Bradfield and Jones (*Trans. Faraday Soc.*, 1941, **37**, 726).

TABLE VI.

Relative effects of R *in ethers*, $RO{\cdot}C_6H_4{\cdot}Cl$ *on the rate of bromination in* 75% *acetic acid.*

R =	$C_6H_5{\cdot}CH_2$.	Me.	Et.	Pr^n.	Pr^i.
p-Series	55	100	255	336	837
o-Series	—	100	254	289	546

The work described above was carried out at the University College of N. Wales, and much of the present discussion was incorporated in a thesis by one of us (G. I. D., University of Wales, 1931). The authors wish to express their thanks to Professor Gwyn Williams for friendly and critical discussion of the present manuscript.

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297. *The Synthesis of Benziminazoles from* ortho-*Phenylenediamines and Imino-ethers.*

By F. E. KING and R. M. ACHESON.

A synthesis of benziminazoles from imino-ethers and *o*-phenylenediamines, used by Wheeler (*Amer. Chem. J.*, 1895, **17**, 397) to prepare 2-phenylbenziminazole, has been further investigated, and the optimum reaction conditions have been determined. The method has been applied to *N*-alkylbenziminazoles, *e.g.*, 6-*chloro*-2-*methyl*-1-(3′-*diethylaminopropyl*)*benziminazole*, but a side reaction leading to amidines of the type (II) limits the formation of analogues containing a 5-chloro-substituent.

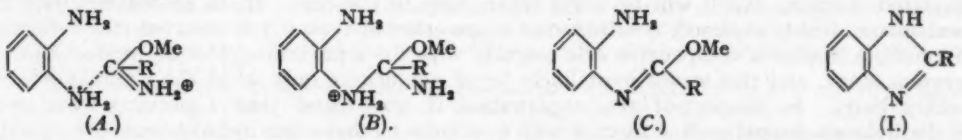
THE condensation of ethylenediamines with imino-ether hydrochlorides is a well-established method for the preparation of dihydroglyoxalines (*e.g.*, dimethyl penillate-G, Merck, C.P.S., 365) and takes place under mild conditions with considerable ease (see *Chem. Zentr.*, 1929, II, 2970; Klarer and Urech, *Helv. Chem. Acta*, 1944, **27**, 1762; Delaby and Harispe, *Bull. Soc. chim.*, 1944, [v], **11**, 277). An interest in the synthesis of certain benziminazoles, unobtainable by the usual comparatively drastic methods owing to the presence of labile substituents, led us to investigate the analogous reaction between *o*-phenylenediamines and some simple imino-ethers.

Wheeler (*Amer. Chem. J.*, 1895, **17**, 397) has already prepared 2-phenylbenziminazole from *o*-phenylenediamine and benzimino methyl ether, and the corresponding phenylbenzoxazole from *o*-aminophenol. It is reported that, without heating, little if any reaction occurs, but no yields are given to indicate the efficacy of the synthesis, and further examples of its application appear not to have been described.

Experiments with phenacetimino methyl ether and *o*-phenylenediamine have confirmed the importance of temperature in this reaction. Heating the reagents in methanol failed to induce appreciable cyclisation (cf. Ashley *et al.*, J., 1942, 103), but when they were heated alone at 115° slow evolution of ammonia was observed which became rapid above 130°, and 2-benzylbenziminazole was obtained in 35% yield. On the other hand, with the addition of small quantities of the imino-ether hydrochloride a perceptible reaction occurred in boiling methanol, and when cold methyl-alcoholic solutions containing equivalent amounts of the imino-ether hydrochloride and *o*-phenylenediamine were mixed the heat of reaction caused the solvent to boil and the yield of benziminazole reached 84%. In presence of two equivalents of acid, the yield of benziminazole was again high, but with three equivalents, and even after prolonged heating, the formation of benziminazole was approximately only 50%. Similar results were obtained with *N*-methyl-*o*-phenylenediamine, leading to 1-*methyl*-2-*benzylbenziminazole* which was conveniently isolated as a *picrate*. Phenacetimino benzyl thioether hydrochloride readily formed benziminazoles with both *o*-diamines, but the use of *o*-phenylenediamine dihydrochloride again resulted in a diminished yield of the 2-benzylbenziminazole.

The retardation caused by a total of three equivalents of acid is readily explained in terms of the following scheme, in which the affinity of the imino-ether cation for the unshared electrons of either aromatic amino-group initiates the reaction. Formation of the complex (*B*)

is followed by loss of an ammonium ion giving a substituted imino-ether (*C*) capable of intramolecular condensation to the benziminazole (I) by elimination of methanol. Conversion of the *o*-diamine into a diquaternary salt hinders the process by removing the attractive force leading to the complex (*B*).



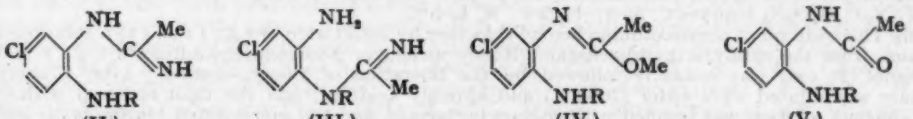
(*A*.) (*B*). (*C*.) (I.)

Stages (*A*)—(*C*) of this scheme are identical with those involved in the synthesis of *N*-substituted imino-ethers, *e.g.*, OEt·CR:N·CH_2·CO_2Et, from the simple imino-compounds OEt·CR:NH and glycine ethyl ester hydrochloride (Schmidt, *Ber.*, 1914, **47**, 2545; Cornforth and Cornforth, *J.*, 1947, 96).

o-Aminophenol and the hydrochlorides of acetimino methyl ether and of the analogous thiobenzyl compound also reacted easily giving 2-methylbenzoxazole, which was identified by its picrate. In view of the mechanism proposed for these reactions it is probable that the condensation of imino-ether hydrochlorides with ethanolamines will yield oxazolines. This conclusion is at variance with the observation of Drozov and Bekhli (*J. Gen. Chem. Russia*, 1944, **14**, 480) that the compound obtained by the action of palmitimino ethyl ether hydrochloride on 3-piperidino-2-hydroxy-*n*-propylamine is a dihydroglyoxaline, but the recorded analytical figures for the picrate, by which the product was characterised, do not altogether exclude the oxazoline structure.

In addition to the purpose for which it was originally intended, the imino-ether method was also of interest in connexion with the synthesis of some *N*-dialkylaminoalkylbenziminazoles. Compounds of this type so far prepared (see King, Beer, and Waley, *J.*, 1946, 92) are without plasmodicidal properties, but those tested do not contain the chlorinated aromatic nucleus characteristic of most successful synthetic antimalarials. Three chloro-derivatives representative of the series of basically-substituted benziminazoles were therefore selected for synthesis, *viz.*, 5-chloro-, 6-chloro-, and 5 : 6-dichloro-2-methyl-1-(3′-diethylaminopropyl)benziminazole. The necessary monochloro-*o*-diamines were obtained from the chloro-*o*-nitroanilines (King and Acheson, *J.*, 1946, 811; King, Acheson, and Yorke-Long, *J.*, 1948, 1926) by catalytic reduction; their cyclisation to benziminazoles was first carried out with acetic acid by heating in 4N-hydrochloric acid, the method of Phillips (*J.*, 1928, 2393). The 5 : 6-*dichlorobenziminazole* was similarly prepared, the requisite diamine being obtained by condensation of 1 : 2-dichloro-4 : 5-dinitrobenzene with 3-diethylaminopropylamine and reduction of the resulting 4 : 5-dichloro-2-nitro-*N*-(3′-diethylaminopropyl)aniline. The products were either straw-coloured oils or low-melting solids forming stable *dipicrates* and *dipicrolonates*.

The preparation of these benziminazoles by the imino-ether method was fully successful only when applied to 5-chloro-2-amino-*N*-(3′-diethylaminopropyl)aniline, which readily combined with acetimino benzyl thioether hydrochloride to give the 6-chlorobenziminazole in good yield. 4-Chloro-2-amino-*N*-(3′-diethylaminopropyl)aniline and acetimino methyl ether, on the other hand, gave only a small amount of the required benziminazole, isolated as the dipicrate. The principal product separated as a *hydrochloride*, and from analyses appeared to be an amidine for which structures (II) and (III) are possible. The base, a distillable but easily oxidisable oil, was characterised by *mono-* and *di-picrates*. These derivatives did not analyse satisfactorily for nitrogen, and other possible structures were considered, *e.g.*, the imino-ether (IV) and its hydrolysis product (V), but these alternatives do not appear to be feasible. The results of



(II.) (III.) (IV.) (V.)

R = ·$[CH_2]_3$·NEt_2

attempted diazotisation and coupling with alkaline β-naphthol, although not conclusive, appear to favour structure (II). The amidine gave the corresponding 5-chlorobenziminazole in nearly theoretical amount on boiling it with acetyl chloride. All attempts to acetylate 4-chloro-2-nitro-*N*-(3′-diethylaminopropyl)aniline, in the hope of preparing an isomer of (V) for comparative purposes, were unsuccessful.

A *base* similar to that regarded as (II) was the principal product when acetimino methyl ether hydrochloride reacted with 4 : 5-dichloro-2-amino-*N*-(3′-diethylaminopropyl)aniline.

The ready formation of the 6-chlorobenziminazole, but not of the 5-chloro- and 5 : 6-dichlorobenziminazoles, suggests that ease of ring-closure may depend on the relative basicity of the alkylated *N*-atom, which will be lower when *para* to chlorine. If, in accordance with the mechanism already outlined, a substituted imino-ether of type (*C*) is involved, the formation of amidines implies a competitive side reaction with the ammonium chloride produced in the previous stage, and this would most likely be of significance only when the *o*-amino-group is weakly basic. In support of this explanation it was found that 4-methoxy-2-amino-*N*-(3′-diethylaminopropyl)aniline reacted with acetimino methyl ether hydrochloride giving without difficulty 5-methoxy-2-methyl-1-(3′-diethylaminopropyl)benziminazole, which was also synthesised for comparison by the method of Phillips.

Experimental.

2-Benzylbenziminazole (I; R = CH_2Ph).—(i) When *o*-phenylenediamine (0·54 g., 1 mol.) and phenylacetimino methyl ether (0·75 g., 1·1 mols.) were heated under reflux in methyl alcohol (3 c.c.) for 1 hour, there was no evolution of ammonia or separation of product on cooling and seeding with 2-benzylbenziminazole. Heating alone for 2½ hours, finally at 150—160°, and crystallisation from ethanol (charcoal) gave the benziminazole as a dark brown solid, m. p. 183—184° (35%).

(ii) Boiling the reactants under reflux in methanol for 6 hours after the addition of a minute amount of the imino-ether hydrochloride gave a product (26%) of m. p. and mixed m. p. 186°. When cold solutions of the diamine (2·16 g., 1 mol.) and of the imino-ether hydrochloride (3·71 g., 1 mol.) in methanol (8 c.c. and 4 c.c., respectively) were mixed, the liquid boiled and ammonium chloride separated. Pouring into water (100 c.c.) gave 2-benzylbenziminazole (3·5 g., 84%), crystallising from aqueous alcohol in colourless needles, m. p. 191° (Found after drying at 100° in a vacuum: C, 80·6; H, 5·8. Calc. for $C_{14}H_{12}N_2$: C, 80·8; H, 5·8%).

(iii) A mixture of the diamine (1·08 g., 1 mol.), imino-ether hydrochloride (1·86 g., 1 mol.), and hydrogen chloride (0·37 g., 1 mol.) in methanol (6 c.c.), heated under reflux for 1 hour, poured into water, and basified, gave the benziminazole (1·65 g., 79%), m. p. 190°. A similar experiment in which a further equivalent of hydrogen chloride was added gave, after boiling for 1 hour, 53% of the benziminazole, m. p. 188—189°, and further heating failed to increase the yield.

(iv) Solutions of *o*-phenylenediamine (2·16 g., 1 mol.) and phenacetimino benzyl thioether hydrochloride (5·56 g., 1 mol.) in methanol (8 c.c. and 8 c.c., respectively) developed a strong odour of toluene-ω-thiol on mixing. After 20 minutes the solution was poured into water (350 c.c.) containing concentrated hydrochloric acid (20 c.c.). The thiol was removed by ether, the aqueous solution basified, and the benzylbenziminazole (3 g., 72%), m. p. 187—188°, collected. When a methyl-alcoholic solution of the imino-thioether hydrochloride and *o*-phenylenediamine dihydrochloride was set aside for 16 hours, the yield of benziminazole, m. p. 187°, was 42%.

2-Methylbenziminazole (I; R = Me).—A mixture of *o*-phenylenediamine (1·08 g.) and acetimino methyl ether hydrochloride (1·1 g.) in methanol (5 c.c.) was warmed on a steam-bath for 10 minutes, and the colourless 2-methylbenziminazole (1·2 g., 91%), m. p. 175—176°, precipitated with water. Phillips (*J.*, 1928, 2393) gives m. p. 176°. The benziminazole picrate separated from ethanol in long thin yellow prisms, m. p. 211—212° (Phillips, *J.*, 1929, 2820, gives m. p. 207—208°) (Found: C, 46·6; H, 3·0. Calc. for $C_8H_8N_2,C_6H_3O_7N_3$: C, 46·5; H, 3·0%).

1 : 2-*Dimethylbenziminazole*.—When acetimino methyl ether hydrochloride (0·65 g., 1 mol.) was added to a solution of *N*-methyl-*o*-phenylenediamine (0·73 g., 1 mol.) in dry methanol (2 c.c.), a vigorous reaction took place, the liquid boiling and a yellow solid being precipitated. After a few minutes the mixture was poured into water (30 c.c.), giving a buff-coloured precipitate (m. p. 66—69°) which, when dried, had m. p. 111—112° (0·52 g., 60%). Phillips (*loc. cit.*, 1929) gives for the trihydrate m. p. 65°, and for the anhydrous compound m. p. 112°. The picrate separated from aqueous ethanol in short yellow needles, m. p. 243° (decomp.) (Found: C, 48·4; H, 3·4. Calc. for $C_9H_{10}N_2,C_6H_3O_7N_3$: C, 48·0; H, 3·5%).

2-Benzyl-1-methylbenziminazole.—(i) Similarly prepared from phenylacetimino methyl ether hydrochloride (0·36 g., 1 mol.) and *N*-methyl-*o*-phenylenediamine (0·24 g., 1 mol.) in methanol (2 c.c.), 2-benzyl-1-methylbenziminazole separated as a pale yellow oil when the reaction mixture was poured into water. This was collected with ether and identified as a *picrate*, which separated from aqueous dioxan in yellowish-brown dodecahedra, m. p. 249° (decomp.) (Found: C, 55·7; H, 3·7; N, 15·9. $C_{15}H_{14}N_2,C_6H_3O_7N_3$ requires C, 55·9; H, 3·8; N, 15·5%).

(ii) The addition of phenacetimino benzyl thioether hydrochloride (6·4 g., 1 mol.) to a solution of the diamine from the catalytic hydrogenation (Raney nickel) of 2-nitromethylaniline (3·5 g., 1 mol.) in methanol (21 c.c.) was instantly followed by the liberation of toluene-ω-thiol. After 2 hours the mixture was diluted with water (200 c.c.) and strongly acidified, and the thiol removed with ether. The aqueous solution was basified with sodium carbonate, and the precipitated *benziminazole* isolated by ether extraction as a straw-coloured oil which on distillation (244—248°/18 mm.) was obtained as a solid (3·4 g., 66%), m. p. 69—70°. After several recrystallisations from *cyclo*hexane it separated in colourless prisms, m. p. 77—78° (Found: C, 80·8; H, 6·2. $C_{15}H_{14}N_2$ requires C, 81·1; H, 6·3%).

2-Methylbenzoxazole.—(i) A mixture of *o*-aminophenol (2·5 g., 1 mol.) and acetimino methyl ether hydrochloride (2·5 g., 1 mol.) in dry methanol (10 c.c.) were heated on a steam-bath for 30 minutes. After the addition of water containing a little sodium carbonate to the semi-solid sludge, the product was collected with ether and distilled. The benzoxazole was obtained as a colourless oil (2·38 g., 78%), b. p. 203—208°, turning red on standing; the *picrate* crystallised from ethanol in yellow prisms, m. p.

117—118° (Found: C, 46·5; H, 2·7; N, 15·2. $C_6H_7ON,C_6H_3O_7N_3$ requires C, 46·4; H, 2·8; N, 15·5%). Wager (*J. Org. Chem.*, 1940, **5**, 133) records m. p. 117—118° but gives no analysis.

(ii) A small rise in temperature was noted when the imino-ether hydrochloride (1·95 g.) and *o*-aminophenol (1·95 g.) were mixed in dry methanol (5 c.c.). After 2 hours at room temperature the mixture was worked up as before, giving 0·7 g. (30%) of benzoxazole, b. p. 200—210°.

(iii) A solution of acetimino benzyl thioether hydrochloride (1 mol.) and *o*-aminophenol (1 mol.) in ethanol was heated on a steam-bath for 20 minutes, cooled, and filtered from the precipitated ammonium chloride. Picric acid was added to the heated filtrate, giving the benzoxazole picrate (51%), m. p. and mixed m. p. 117—118°.

6-Chloro-2-methyl-1-(3′-diethylaminopropyl)benziminazole.—(i) 5-Chloro-2-nitro-*N*-(γ-diethylaminopropyl)aniline (5·0 g.) (King, Acheson, and Yorke-Long, *loc. cit.*) was reduced catalytically over Raney nickel in methanol, and, after filtration and treatment with concentrated hydrochloric acid (5 c.c.), the solution was evaporated to dryness. The residue was heated under reflux with glacial acetic acid (1·1 c.c.) and 4N-hydrochloric acid (25 c.c.) for 90 minutes, and the deep red solution basified and extracted with ether. Distillation of the extract gave 6-chloro-2-methyl-1-(3′-diethylaminopropyl)-benziminazole (3·23 g., 66%) as a thick yellow oil, b. p. 140° (bath temperature)/0·14 mm. The *dipicrate* crystallised from aqueous ethanol in yellow needles, m. p. 217° (decomp.) (Found: C, 43·8; H, 4·1; N, 17·1. $C_{15}H_{22}N_3Cl,2C_6H_3O_7N_3$ requires C, 43·9; H, 3·8; N, 17·1%). The *dihydrochloride*, precipitated by passing hydrogen chloride into a solution of the free base in dry ether, separated in colourless deliquescent prisms, m. p. 100—110° (decomp.) (Found: Cl, 26·9. $C_{15}H_{22}N_3Cl,2HCl$ requires Cl, 30·2%).

(ii) The solution of 5-chloro-2-amino-(γ-diethylaminopropyl)aniline obtained from the nitro-amine (4·52 g.) by catalytic reduction in methanol (20 c.c.) was mixed with acetimino benzyl thioether hydrochloride (3·40 g.) dissolved in methanol (5 c.c.). Next day the solvent was evaporated, the residue treated with aqueous hydrochloric acid, and the benzyl mercaptan removed with ether. Addition of alkali to the aqueous solution and ether extraction gave the *benziminazole* as a pale yellow oil, b. p. 140° (bath temperature)/0·14 mm. (2·1 g., 47%) Found: C, 64·1; H, 8·0; N, 15·0. $C_{15}H_{22}N_3Cl$ requires C, 64·4; H, 7·9; N, 15·0%). The *dipicrolonate*, irregularly-shaped yellow prisms from aqueous ethanol, had m. p. 236° (decomp.) (Found: C, 52·2; H, 5·0; N, 19·3; Cl, 4·4. $C_{15}H_{22}N_3Cl,2C_{10}H_8O_5N_4$ requires C, 52·0; H, 4·7; N, 19·1; Cl, 4·4%). The dipicrate had m. p. and mixed m. p. with a sample prepared by method (i), 217° (decomp.).

5-Chloro-2-methyl-1-(3′-diethylaminopropyl)benziminazole.—4-Chloro-2-nitro-(3′-diethylaminopropyl)-aniline (2 g., 1 mol.) (King and Acheson, *loc. cit.*) was hydrogenated in methanol (15 c.c.) over Raney nickel, and the filtered solution acidified with concentrated hydrochloric acid (3 c.c.) and evaporated to dryness under diminished pressure. The residual oil was heated under reflux with glacial acetic acid (0·75 c.c., 1·9 mol.) in 4N-hydrochloric acid (12·5 c.c.) for 2 hours (cf. Phillips, *J.*, 1928, 2393), and the deep-red solution basified and extracted with ether. The *benziminazole* was a thick yellow oil, b. p. 160—164°(bath temperature)/0·14 mm., which solidified to long needles (1·55 g., 79%), m. p. 53—54° (Found: C, 64·1; H, 7·9; N, 15·3. $C_{15}H_{22}N_3Cl$ requires C, 64·4; H, 7·9; N, 15·0%).

The monopicrate crystallised from ethanol in very small yellow needles, m. p. 165°, but no satisfactory analyses were obtained for this compound owing to its tendency to disproportionate into the free base and the much less-soluble *dipicrate*. The latter salt separated from aqueous ethanol in brown diamond-shaped plates, m. p. 239° (decomp.), with a green lustre (Found: C, 43·6; H, 4·1; N, 17·3; Cl, 4·2. $C_{15}H_{22}N_3Cl,2C_6H_3O_7N_3$ requires C, 43·9; H, 3·8; N, 17·1; Cl, 4·8%).

N-(5-Chloro-2-3′-diethylaminopropylaminophenyl)acetamidine (II).—(i) The nitro-amine (1·27 g., 1 mol.) was catalytically hydrogenated in methanol (10 c.c.) and treated with acetimino methyl ether hydrochloride (0·5 g., 1 mol.). The mixture darkened, and a precipitate (0·84 g., 57%, m. p. 200°); soon formed which was collected after 4½ hours. (Similar results were obtained when the methanol solution was vigorously boiled for 90 minutes.) The filtrate, which gave no further precipitate when concentrated, was basified, and the resulting dark oil collected with ether and distilled. The pale-yellow product (0·25 g.) b. p. 165—170°(bath temperature)/0·15 mm., did not solidify on seeding with the 5-chlorobenziminazole and was evidently a mixture (Found: C, 62·2; H, 8·2%). Treatment with alcoholic picric acid gave a red semi-solid precipitate from which some benziminazole dipicrate, m. p. and mixed m. p. 237° (decomp.), was isolated by fractionation from alcohol. A further portion of the oil gave with alcoholic picrolonic acid the substituted 5-chlorobenziminazole *dipicrolonate*, yellow prisms (from ethanol), m. p. 235—236° (decomp.) (Found: C, 52·1; H, 4·7; Cl, 4·6. $C_{15}H_{22}N_3Cl,2C_{10}H_8O_5N_4$ requires C, 52·0; H, 4·7; Cl, 4·4%). The above precipitate of m. p. 200° crystallised from methanol in colourless prisms, m. p. 204°. It gave no ammonia with aqueous sodium hydroxide, even when heated, and apparently consisted of *N-(5-chloro-2-3′-diethylaminopropylaminophenyl)acetamidine hydrochloride* (Found: C, 53·8; H, 7·9; Cl, 21·6; N, 15·0. $C_{15}H_{25}N_4Cl,HCl$ requires C, 54·0; H, 7·8; Cl, 21·3; N, 16·8%). The base was a pale yellow oil, b. p. 160° (bath temperature)/0·2 mm. (Found: N, 17·7. $C_{15}H_{25}N_4Cl$ requires N, 18·9). A *monopicrate* obtained by the action of aqueous sodium picrate on the aqueous hydrochloride, separated from aqueous ethanol as the dihydrate in very deep-red prisms, m. p. 113—114° (Found: C, 45·2; H, 5·5; N, 16·8. $C_{15}H_{25}N_4Cl,C_6H_3O_7N_3,2H_2O$ requires C, 44·9; H, 5·7; N, 17·4%. Found, after drying over phosphoric anhydride in a vacuum at room temperature: C, 46·1; H, 5·4. $C_{15}H_{25}N_4Cl,C_6H_3O_7N_3,H_2O$ requires C, 46·4; H, 5·5%. Found, after drying at 100° in a vacuum: C, 46·9; H, 5·2; loss 1·9. $C_{15}H_{25}N_4Cl,C_6H_3O_7N_3,\frac{1}{2}H_2O$ requires C, 47·1; H, 5·2; loss 1·7%). The *dipicrate*, short yellow prisms from alcoholic picric acid, had m. p. 156° (decomp.) (Found: Cl, 5·1; $C_{15}H_{25}N_4Cl,2C_6H_3O_7N_3$ requires Cl, 4·7%).

In a further experiment the reduction product of the nitro-compound (2·1 g., 1 mol.) was treated with ethanolic hydrogen chloride (0·3 g. of HCl; 1·1 mols.) before addition to the imino-ether hydrochloride (0·8 g., 1 mol.). A rise of temperature occurred on mixing the reactants, and next day the precipitated hydrochloride of the acetamidine (m. p. 202°; 0·65 g., 27%) was collected, and the filtrate evaporated nearly to dryness. Ammonium chloride (0·2 g., 52%) then separated, and addition of alkali and extraction with ether gave a yellow oil, b. p. 165—170° (bath temperature)/0·2 mm. This failed to crystallise, but

gave, with picric acid, a salt from which benziminazole dipicrate was isolated having m. p. and mixed m. p. 237° (decomp.). When excess (2 mols.) of hydrogen chloride was used, no heating took place on mixing the reactants. Next day, on working up as before, a pale yellow oil, b. p. 160° (bath temperature)/0·2 mm.(1·47 g., 68%), was obtained, apparently the acetamidine (II) (Found : C, 60·9; H, 8·6; N, 17·5. $C_{15}H_{25}N_4Cl$ requires C, 60·7; H, 8·4; N, 18·9%); it was further characterised by the formation of the mono- and di-picrate and the hydrochloride, m. p.s and mixed m. p.s 113—114°, 156° (decomp.), and 202—203°, respectively. A solution of the amidine in hydrochloric acid became deep-cherry red on treatment with sodium nitrite; addition of this solution to alkaline β-naphthol produced a reddish-brown opalescence identical with that formed on making alkaline with sodium hydroxide alone.

Ring-closure of *N*-(5-chloro-2-3′-diethylaminopropylaminophenyl)acetamidine to 5-chloro-2-methyl-1-(3′-diethylaminopropyl)benziminazole was effected by heating the hydrochloride (0·3 g.) with acetyl chloride (3·5 c.c.) under reflux for 3 hours. The solid dissolved, and, after reaction, the mixture was cooled, diluted with water, basified, and extracted with ether. Evaporation of the dried extract gave a yellow oil solidifying to long needles (0·24 g., 92%), m. p. 52—54°, not depressed by addition of pure benziminazole prepared above. The dipicrate had m. p. and mixed m. p. 238° (decomp.) (Found : C, 44·0; H, 3·9; Cl, 5·3%).

4 : 5-*Dichloro*-2-*nitro*-N-(3′-*diethylaminopropyl*)*aniline*.—A mixture of 4 : 5-dichloro-1 : 2-dinitrobenzene (5 g.), freshly fused sodium acetate (6 g.), and 3-diethylaminopropylamine (5·2 c.c.) was heated, after the vigorous initial reaction had subsided, to 160° for 2 hours. The dark-coloured reaction mixture was then diluted with excess of dilute hydrochloric acid, unchanged nitro-compound removed with ether, the aqueous solution basified, and product extracted with ether. (Unless a large volume of water is used, the amine *hydrochloride* separates.) The amine was an orange-red oil (5·08 g., 75%), b. p. 185—190° (bath temperature)/0·02 mm.; its hydrochloride separated from aqueous hydrochloric acid as a microcrystalline yellow powder, m. p. 216—217° (Found : C, 43·5; H, 5·7. $C_{13}H_{19}O_2N_3Cl_2,HCl$ requires C, 43·7; H, 5·6%). The *picrate*, fine yellow needles from aqueous ethanol, had m. p. 171—172° (Found, after drying at 116° in a vacuum : C, 41·6; H, 3·9; N, 15·5; Cl, 12·9. $C_{13}H_{19}O_2N_3Cl_2,C_6H_3O_7N_3$ requires C, 41·5; H, 4·0; N, 15·3; Cl, 12·9%).

5 : 6-*Dichloro*-2-*methyl*-1-(3′-*diethylaminopropyl*)*benziminazole*.—The above nitro-amine (3·82 g., 1 mol.) was reduced, acidified with concentrated hydrochloric acid (3·6 c.c.), and converted into the *benziminazole* as described for the monochloro-derivatives, using acetic acid (0·8 c.c., 1·2 mols.) and 4N-hydrochloric acid (25 c.c.). The product, a pale yellow oil (2·27 g., 61%), b. p. 175—180°(bath temperature)/0·02 mm. (Found : C, 57·1; H, 6·9; N, 13·4; Cl, 22·4. $C_{15}H_{21}N_3Cl_2$ requires C, 57·3; H, 6·7; N, 13·9; Cl, 22·6%), gave a *dipicrate*, long thin needles (from aqueous ethanol), m. p. 226—228° (decomp.) (Found : C, 42·3; H, 3·5; Cl, 8·7. $C_{15}H_{21}N_3Cl_2,2C_6H_3O_7N_3$ requires C, 41·9; H, 3·5; Cl, 9·2%).

When the *o*-diamine, dissolved in methanol containing 1 or 2 equivalents of hydrochloric acid, was treated with acetimino methyl ether hydrochloride in the manner of the previous experiments, the resulting base did not form purifiable salts with picric or picrolonic acid. From its composition the pale yellow oil, b. p. 180—185°(bath temperature)/0·2 mm., is probably N-(4 : 5-*dichloro*-2-3′-*diethylaminopropylaminophenyl*)*acetamidine* (Found : C, 54·6, 54·2; H, 7·4, 7·5; N, 15·0. $C_{15}H_{24}N_4Cl_2$ requires C, 54·4; H, 7·3; N, 16·1%).

5-*Methoxy*-2-*methyl*-1-(3′-*diethylaminopropyl*)*benziminazole*—2-Nitro-4-methoxy-*N*-(3′-diethylaminopropyl)aniline (7·0 g.) (King and Acheson, *J.*, 1946, 811) was hydrogenated in methanol over Raney nickel, the solution was filtered, and the filtrate and washings (54 c.c.) were divided into two parts. (i) To one half, concentrated hydrochloric acid (4 c.c.) was added, and the gum obtained on evaporation was heated under reflux with acetic acid (1 c.c.) and 4N-hydrochloric acid (15 c.c.). The methoxybenziminazole, isolated in the usual way, was a pale-yellow oil (2·2 g., 64%), b. p. 186—188°/0·8 mm., which formed long prisms, m. p. *ca.* 40° (Simonov, *Chem. Abs.*, 1941, **35**, 2870, gives b. p. 184—185°/2 mm. but no m. p.) (Found : C, 70·0; H, 9·2; N, 15·2. Calc. for $C_{16}H_{25}ON_3$: C, 69·8; H, 9·1; N, 15·3%). The dipicrate separated from aqueous *n*-propanol in clusters of short yellow prisms, m. p. 238—239° (decomp.) after sintering at *ca.* 225° (Simonov, *loc. cit.*, gives m. p. 236°) (Found : C, 46·1; H, 4·4. Calc. for $C_{16}H_{25}ON_3,2C_6H_3O_7N_3$: C, 45·8; H, 4·2%). (ii) Acetimino methyl ether hydrochloride (1·4 g.) was added to the other half of the triamine solution, which after 30 minutes was largely evaporated on a steam-bath. When alkali was added, ammonia was evolved, and extraction with ether gave an oily base (2·0 g.) distilling at 178—188°/0·8 mm. This base failed to solidify on seeding, but, when treated with alcoholic picric acid, gave the benziminazole dipicrate (2·8 g.), m. p. 229° (decomp.) (after one recrystallisation from *n*-propanol) (Found : C, 46·2; H, 4·5%).

5-Chloro-, 6-chloro-, and 5 : 6-dichloro-2-methyl-1-(3′-diethylaminopropyl) benziminazole proved to be inactive against *Plasmodium relictum* infection in chicks. For these tests we are indebted to Miss A. Bishop, Molteno Institute, University of Cambridge. We also thank the Medical Research Council for the award (to R. M. A.) of a Studentship.

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298. *Benziminazoles related to Pteroic and Pteroylglutamic Acids.*

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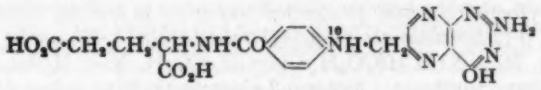
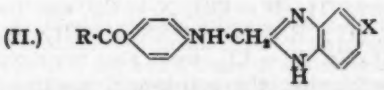
Benziminazole analogues of pteroic and pteroylglutamic acids have been prepared by condensing 2-chloromethylbenziminazole with *p*-aminobenzoic acid or its ethyl ester and with ethyl *p*-aminobenzoyl-L-glutamate. The intermediate *p*-nitrobenzoylglutamic ester was readily obtained in an optically pure condition from *p*-nitrobenzoyl chloride and ethyl L-glutamate, and the preparation of the corresponding acid from L-glutamic acid has been improved.

Several investigations on the effect on biological activity of modifications in the structure of pteroylglutamic acid (folic acid) (I) have now been reported. These mutations include the replacement in (I) of (*a*) the 4-hydroxyl by an amino-group (Seeger, Smith, and Hultquist, *J.*

(I.) 

(II.) 

Amer. Chem. Soc., 1947, **69**, 2567), (*b*) the 7-hydrogen atom by methyl (Martin, Tolman, and Moss, *Arch. Biochem.*, 1947, **12**, 318; Franklin, Stokstad, Belt, and Jukes, *J. Biol. Chem.*, 1947, **169**, 427), (*c*) the L-glutamic acid residue by L-aspartic acid (Hutchings *et al.*, *ibid.*, 1947, **170**, 323), and (*d*) of H_{10} by formyl (Gordon, Ravel, Eakin, and Shrive, *J. Amer. Chem. Soc.*, 1948, **70**, 878) and by methyl and phenacyl groups (Cosulich and Smith, *ibid.*, p. 1922). Woolley and Pringle (*J. Biol. Chem.*, 1948, **174**, 327) have described an *iso*xanthopterin-6-carboxyamidobenzoylglutamic acid, and more remote analogues of (I), *e.g.*, quinoxaline-2-carboxyamidobenzoylglutamic acid (Woolley and Pringle, *loc. cit.*) and a *p*-(4-quinazolyl)aminobenzoyl-L-glutamic acid (Martin, Moss, and Avakian, *ibid.*, 1947, **167**, 737), have also been prepared.

In general, these compounds are in varying degrees pteroylglutamic acid antagonists for several species, but the 10-formyl derivative of (I)—as would be expected from its constitution as a glutamyl derivative of the *S. fæcalis* R. factor (rhizopterin) (Wolf *et al.*, *J. Amer. Chem. Soc.*, 1947, **69**, 2753)—is a potent growth promoter for certain micro-organisms. The quinazoline is also said to have slight stimulating properties, but the observation requires confirmation in view of its exceptional nature.

The following account gives details of the synthesis of some benziminazoles structurally related to pteroylglutamic acid. While these compounds were undergoing microbiological examination, a communication appeared from Edwards, Starling, Mattocks, and Skipper (*Science*, 1948, **107**, 119) describing the results of experiments with [II; R = $NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$, X = H], but no particulars of their method of preparation have yet been published.

The required benziminazoles were expected to be available through the imino-ether synthesis (King and Acheson, preceding paper), but the preparation of the necessary imino-ethers from cyanides of the type $R{\cdot}C_6H_4{\cdot}NH{\cdot}CH_2{\cdot}CN$ could not be accomplished on account of difficulties due to the presence of the secondary amino-group. However, a satisfactory alternative was found in the use of chloroacetimino ethyl ether hydrochloride, which very readily condensed with *o*-phenylenediamine giving 2-chloromethylbenziminazole, identical with that obtained from chloroacetic acid and the *o*-diamine by heating in hydrochloric acid (Bloom and Day, *J. Org. Chem.*, 1939, **4**, 14). The chloromethyl compound slowly reacted in boiling alcohol with ethyl *p*-aminobenzoate, forming the *hydrochloride* of *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoate*, which was hydrolysed with hot 2N-alkali to the *acid* (II; R = OH, X = H), also obtained from the chloromethylbenziminazole and *p*-aminobenzoic acid.

It was not possible to synthesise the glutamic acid derivative [II; R = $NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$, X = H] from the above acid, the insolubility of the acid chloride rendering it inactive towards aqueous alkaline solutions of glutamic acid and also of glycine. The acylation of glycine with p-(2-*benziminazolyl*)*methylaminobenzazide* under similar conditions was also unsuccessful, apparently for the same reason. The necessary azide was obtained from 1-(*p*-aminobenzoyl)-2-benzylidenehydrazine, $CHPh{:}N{\cdot}NH{\cdot}CO{\cdot}C_6H_4{\cdot}NH_2$ (Curtius, *J. pr. Chem.*, 1917, **45**, 336), and 2-chloromethylbenziminazole in boiling alcohol, followed by the action of hydrochloric acid (which removed the protecting benzylidene group) and sodium nitrite.

An attempt was therefore made to synthesise the pteroylglutamic acid analogue from 2-chloromethylbenziminazole and *p*-aminobenzoyl-L-glutamic acid, the latter being obtained by catalytic reduction of the corresponding *p*-nitro-compound. The acylation of L-glutamic acid was carried out in sodium hydroxide solution, to avoid racemisation (cf. Carter and Stevens, *J. Biol. Chem.*, 1941, **138**, 627), and the use of only one equivalent of *p*-nitrobenzoyl chloride dissolved in dioxan was sufficient to give 70—80% of fairly pure *p*-nitrobenzoyl-L-glutamic acid. The *p*-aminobenzoylglutamic acid failed, however, to give a crystalline derivative with 2-chloromethylbenziminazole, but the synthesis of p-(2-*benziminazolyl*)*methylaminobenzoyl-L-glutamic acid* was finally achieved from ethyl *p*-aminobenzoylglutamate. This was easily obtained without any detectable racemisation by boiling a solution of *p*-nitrobenzoyl chloride and the hydrochloride of ethyl glutamate in benzene, the resulting *ethyl* p-*nitrobenzoyl-L-glutamate* being catalytically reduced. The condensation of ethyl *p*-aminobenzoyl-L-glutamate with 2-chloromethylbenziminazole proceeded smoothly in boiling ethanol, giving a *hydrochloride* from which *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoyl-L-glutamate* was obtained and then hydrolysed to [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = H] by cold alkali.

By very similar methods, *5-chloro-2-chloromethylbenziminazole*, p-(*5-chloro-2-benziminazolyl*)*methylaminobenzoic acid* (II; R = OH, X = Cl), and the corresponding L-glutamic *acid* and *ethyl* ester derivatives, [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = Cl] and [II; R = NH·CH(CO_2Et)·CH_2·CH_2·CO_2Et, X = Cl], were also prepared. Some analogous compounds were prepared from DL-methionine, the amino-acid reacting with *p*-nitrobenzoyl chloride in aqueous alkali under the usual conditions to give p-*nitrobenzoyl-DL-methionine*. The nitro-compound was reduced by ferrous hydroxide to p-*aminobenzoyl-DL-methionine*, which was condensed with 2-chloromethylbenziminazole to the *product* [II; R = NH·CH(CO_2H)·CH_2·CH_2·SMe, X = H] without the necessity for proceeding through the ester.

At one time in the course of this work some experiments were made on the synthesis of compounds of type (II) from arylaminoacetyl-*o*-phenylenediamines, Ar·NH·CH_2·CO·NH·C_6H_4·NH_2. *4-Chloro-2-nitrochloroacetanilide* was prepared from chloroacetyl chloride and 4-chloro-2-nitroaniline, and the product condensed with aniline. In boiling *n*-propanol, *anilinoaceto-4-chloro-2-nitroanilide* was obtained, but with pyridine as solvent the principal product was *4-chloro-2-nitroanilinoacetylpyridinium chloride*. The *o*-nitroanilide was hydrogenated over Raney nickel, and on heating the resulting *anilinoaceto-4-chloro-2-aminoanilide* under reflux with aqueous methanolic hydrogen chloride, N-(*5-chloro-2-benziminazolyl*)*methylaniline* was obtained, and characterised as a *picrate*. The synthesis proved much less satisfactory with the feebly reactive ethyl *p*-aminobenzoate, the condensation with 2-nitrochloroacetanilide in boiling propanol (which required pyridine as a catalyst) giving only 12% of p-*carbethoxyanilinoaceto-2-nitroanilide*. Catalytic reduction led to the corresponding 2-*amino*-compound, which was cyclised in boiling alcoholic acid to a hydrochloride identical with that synthesised from ethyl *p*-aminobenzoate and 2-chloromethylbenziminazole. The picrate of the benziminazole (II; R = OEt, X = H) was obtained merely on heating the *o*-diamine with alcoholic picric acid, but the poor yield obtained at the intermediate stage renders the alternative synthesis preferable, and these experiments were discontinued.

Methyl p-*nitrobenzoyl-L-glutamate* and 2-(*2'-chloroethyl*)*benziminazole hydrochloride* were also prepared in the course of this work.

Experimental.

2-Chloromethylbenziminazole.—Solutions of chloroacetiminoethyl ether hydrochloride (4·3 g., 1 mol.) and of *o*-phenylenediamine (3 g., 1 mol.) in dry ethanol (total 20 c.c.) developed heat on mixing, and ammonium chloride separated. After several hours, the mixture was diluted with water, and the precipitate (3 g., 70%) crystallised from dioxan to give colourless needles, m. p. 162°, identical with the product obtained by Bloom and Day's method (*loc. cit.*).

Ethyl p-(*2-Benziminazolyl*)*methylaminobenzoate.*—A solution in ethyl alcohol (100 c.c.) of 2-chloromethylbenziminazole (17·8 g., 1 mol.), ethyl *p*-aminobenzoate (17·8 g., 1 mol.), and a small quantity of sodium iodide was heated under reflux for 10 hours. The solid (25·5 g., 72%) which then slowly separated consisted of the *hydrochloride* of ethyl *p*-(2-benziminazolyl)methylaminobenzoate which, after being washed with a little alcohol, crystallised from ethanol or water in bush-like clusters of colourless prisms, m. p. 255° (decomp.) (Found: C, 61·2; H, 5·4; N, 13·0. $C_{17}H_{17}O_2N_3$,HCl requires C, 61·5; H, 5·4; N, 12·7%). Warm alcoholic solutions of the hydrochloride and picric acid deposited overnight the *picrate* in golden-yellow polyhedra, m. p. 210° (decomp.) (Found: C, 52·6; H, 3·9; N, 15·6. $C_{17}H_{17}O_2N_3$,$C_6H_3O_7N_3$ requires C, 52·7; H, 3·8; N, 16·0%). The addition of ammonia to a hot aqueous solution of the hydrochloride liberated the *amino*-ester, crystallising from ethanol in colourless platelets, m. p. 248° (decomp.) (Found: C, 68·7; H, 5·9. $C_{17}H_{17}O_2N_3$ requires C, 69·1, H, 5·8%).

p-(*2-Benziminazolyl*)*methylaminobenzoic Acid.*—(i) A mixture of 2-chloromethylbenziminazole (8·5 g., 1 mol.) and *p*-aminobenzoic acid (7·0 g., 1 mol.) with a small amount of sodium iodide was heated in ethyl alcohol (55 c.c.) under reflux for 12 hours. After cooling, the product (8·7 g., 57%) was collected,

washed with cold alcohol, and crystallised from N-hydrochloric acid. The resulting *hydrochloride* separated in colourless long prisms, m. p. 268° (decomp.) (Found : C, 58·5; H, 4·8. $C_{15}H_{13}O_2N_3$,HCl requires C, 59·3; H, 4·6%). Its aqueous solution, treated with sodium hydrogen carbonate solution, gave p-(*2-benziminazolyl*)*methylaminobenzoic acid*, which crystallised from nitrobenzene or ethoxyethanol–water in minute elongated prisms, m. p. 281° (decomp.) (Found : C, 66·8; H, 4·7. $C_{15}H_{13}O_2N_3$ requires C, 67·4; H, 4·9%). The addition of alcoholic picric acid to a solution of the hydrochloride in warm water gave a *picrate*, slowly separating on cooling, which crystallised from alcohol in yellow prisms, m. p. 216° (decomp.) (Found : C, 50·4; H, 3·6; N, 16·6. $C_{15}H_{13}O_2N_3,C_6H_3O_7N_3$ requires C, 50·1; H, 3·2; N, 16·9%).

(ii) Ethyl *p*-(2-benziminazolyl)methylaminobenzoate (1·7 g.) was treated with 2N-alcoholic potassium hydroxide (20 c.c.) which was then heated to boiling for 5 minutes. After the addition of water, the cold solution was neutralised with dilute acetic acid, and the precipitated solid was collected and recrystallised from ethoxyethanol–water, giving the *p*-aminobenzoic acid derivative, m. p. 279° (decomp.).

The hydrochloride of the benziminazole (6·8 g.) was dissolved in warm phosphoryl chloride (150 c.c.) which after the addition of phosphorus pentachloride (4·7 g.) was heated under reflux for 20 minutes. Most of the phosphoryl chloride was then evaporated off under reduced pressure, and the gummy residue triturated with anhydrous ether. The product (9·7 g.), a buff powder containing phosphorus, reacted as the acid chloride in giving with ethyl alcohol the above ester, m. p. 255° (decomp.) The bulk (8·5 g.), added during 1½ hours to a solution of L-glutamic acid (3·5 g., 1 mol.) in water (100 c.c.) containing sodium hydrogen carbonate (10 g., 5 mols.) at 0°, was largely converted into a bicarbonate-insoluble product; no further precipitate was obtained on acidifying the solution with acetic acid. Similar negative results were obtained when the supposed acid chloride reacted with a solution of glycine in N-sodium hydroxide.

p-(*2-Benziminazolyl*)*methylaminobenzazide*.—(*p*-Aminobenzoyl) benzylidenehydrazine (4 g.) (Curtius, *loc. cit.*), 2-chloromethylbenziminazole (2·9 g.), and a trace of sodium iodide were heated in refluxing ethanol (110 c.c.) for 15 hours. The colourless solid (4·6 g., 68%) which separated during the reaction was the *hydrochloride* of the compound (II; R = NH·N:CHPh, X = H). When collected and recrystallised from ethanol–water it formed microscopic needles, m. p. 283° (decomp.) (Found : C, 65·1; H, 4·9; N, 17·0. $C_{22}H_{19}ON_5$,HCl requires C, 65·1; H, 4·9; N, 17·2%). With picric acid in aqueous-alcoholic solution a precipitate of the *picrate* was formed, m. p. (after crystallisation from ethoxyethanol–water), 224° (decomp.) (Found, after drying at 110° in a vacuum : C, 54·8; H, 4·0; N, 18·2. $C_{22}H_{19}ON_5,C_6H_3O_7N_3$ requires C, 54·5; H, 4·0; N, 18·2%). A solution of the hydrochloride in 30% alcohol, neutralised with sodium hydrogen carbonate, gave the *benziminazole* (II; R = NH·N:CHPh, X = H), which crystallised from ethoxyethanol–water in colourless minute plates, m. p. 276° (Found : C, 70·7; H, 5·2. $C_{22}H_{19}ON_5$ requires C, 71·5; H, 5·2%).

The hydrochloride (4·5 g.) was dissolved in 2N-hydrochloric (250 c.c.) by heating to boiling for a few minutes. The solution was then cooled, and after removal of the benzaldehyde by ether extraction, was treated at 0° with sodium nitrite (0·9 g.). The gummy precipitate which separated slowly hardened and formed a powder. This was insoluble in the usual solvents, but the product obtained by shaking it with sodium carbonate solution, which was probably the *azide* (II; R = N_3, X = H), dissolved in warm acetone and separated on addition of light petroleum in microscopic long prisms decomposing vigorously at *ca.* 150° (Found : N, 28·0. $C_{15}H_{11}ON_6$ requires N, 28·8%). When this was added to a stirred solution of glycine in excess of sodium carbonate, apparently unchanged azide was recovered.

p-*Nitrobenzoyl-L-glutamic Acid*.—(i) L-Glutamic acid (19·6 g., 1 mol.) was dissolved in N-sodium hydroxide (270 c.c., 2 mols.) which was then stirred at room temperature for 1 hour during the simultaneous addition of a solution (66 c.c.) of *p*-nitrobenzoyl chloride (25 g., 1 mol.) in dioxan and of 2N-sodium hydroxide (66 c.c.). Shortly after, the solution was filtered and then acidified to Congo-red with 5N-hydrochloric acid (*ca.* 55 c.c.). When the precipitated *p*-nitrobenzoic acid had been removed, the filtrate was concentrated under reduced pressure to about 70 c.c., and the crude *p*-nitrobenzoylglutamic acid (33 g., 84%), m. p. *ca.* 110°, was collected after several hours. Crystallisation from four times its weight of water gave the pure acid, m. p. 114—116°, $[\alpha]_D^{19°}$ +16·9° in N-sodium hydroxide (Found : C, 45·4; H, 4·3; N, 8·8. Calc. for $C_{12}H_{11}O_7N_3,H_2O$: C, 45·9; H, 4·5; N, 8·9%).

(ii) Ethyl *p*-nitrobenzoylglutamate (2 g.) obtained by nitrobenzoylation of the glutamic ester (see below) was heated under reflux with 2N-hydrochloric acid (20 c.c.) until dissolved (1½ hours). On cooling, *p*-nitrobenzoic acid separated and was removed after an hour; next day the filtrate had deposited *p*-nitrobenzoylglutamic acid (0·75 g., 45%). Several crystallisations from water gave the pure product, m. p. 114—116°, $[\alpha]_D^{20°}$ +17·5° in N-sodium hydroxide.

Hydrolysis of the ester (11 g.) was also effected in acetone (160 c.c.) by shaking with 5N-sodium hydroxide (40 c.c.) at room temperature for 2 hours. The solution was acidified with hydrochloric acid, and concentrated under reduced pressure to remove acetone, whereupon nitrobenzoylglutamic acid (7·7 g., 83%) was obtained, m. p. (after recrystallisation), 113—115°, $[\alpha]_D^{19}$ +16·9° in N-sodium hydroxide.

Ethyl p-*Nitrobenzoyl-L-glutamate*.—(i) *p*-Nitrobenzoyl-L-glutamic acid (3 g.) was heated under reflux in 5% ethanolic hydrogen chloride for 4 hours. On evaporation of the solvent, the ester remained as a colourless gum which slowly solidified. A solution in warm ethyl acetate, treated with light petroleum, gave a microcrystalline product (1·7 g., 50%), m. p. 89—91° (Found : C, 54·6; H, 5·6; N, 7·6. $C_{16}H_{20}O_7N_2$ requires C, 54·5; H, 5·7; N, 7·9%).

(ii) A suspension of L-glutamic acid (20 g.) in ethanol (150 c.c.) was saturated with dry hydrogen chloride. More anhydrous alcohol (300 c.c.) was then added, and the mixture gently heated under reflux for 3 hours and finally evaporated under reduced pressure. To remove the last traces of alcohol, pure benzene (50 c.c.) was added and distilled. Finally, a solution of *p*-nitrobenzoyl chloride (27 g., 1·05 mols.) in dry benzene was introduced, and the mixture heated under reflux for several hours until evolution of hydrogen chloride had ceased. The benzene solution, cooled and washed with 5% aqueous bicarbonate, was then dried ($CaCl_2$) and evaporated, leaving the *p*-nitrobenzoyl ester as a colourless solid (33 g., 70%) identical when crystallised from 50% alcohol or ether–light petroleum with the product, m. p. 94—95°, obtained by esterification of the acid.

Ethyl p-*Aminobenzoyl-L-glutamate* (cf. Waller *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 20).—The nitro-ester

was hydrogenated in alcoholic solution over palladised charcoal at room temperature and 2—3 atm. The *p*-aminobenzoylglutamate was isolated by evaporating the filtered solution and treatment with water. Crystallisation from a little alcohol gave small prisms, m. p. 140—141° (Found: C, 59·3; H, 6·8; N, 8·5. Calc. for $C_{16}H_{22}O_5N_2$: C, 59·6; H, 6·9; N, 8·9%).

p-*Aminobenzoyl-*L*-glutamic Acid.*—A solution of the nitro-acid (10·8 g.) in ethanol (100 c.c.) was hydrogenated over palladised charcoal at room temperature and 2—3 atm. Evaporation of the filtered solution left a gum, which was dissolved in a little hot water. The aminobenzoylglutamic acid (8·8 g., 90%) crystallising on cooling had m. p. 169—170° and $[\alpha]_D^{22°}$ +29·2° in N-sodium hydroxide.

Ethyl p-(2-*Benziminazolyl*)*methylaminobenzoyl-*L*-glutamate.*—2-Chloromethylbenziminazole (3·5 g.), ethyl *p*-aminobenzoylglutamate (7 g.), and a little sodium iodide in ethanol (25 c.c.) were heated under reflux for 10 hours. The solvent was evaporated (charcoal) to half bulk, and the solution left until next day. The product which had then separated was a *hydrochloride* (3·8 g., 36%), which crystallised from alcohol, containing hydrogen chloride, in minute elongated prisms, m. p. 232° (decomp.) (Found: C, 58·7; H, 5·9; N, 11·5; Cl, 7·4. $C_{24}H_{28}O_5N_4$,HCl requires C, 58·9; H, 5·9; N, 11·5; Cl, 7·3%). The *ethyl* p-(2-*benziminazolyl*)*methylaminobenzoylglutamate*, colourless minute needles from 50% alcohol, had m. p. 125° (Found: N, 12·6. $C_{24}H_{28}O_5N_4$ requires N, 12·4%). The *picrate* separated from alcohol in yellow prisms, m. p. 179° (Found: C, 52·6; H, 4·5; N, 14·5. $C_{24}H_{28}O_5N_4,C_6H_3O_7N_3$ requires C, 52·4; H, 4·5; N, 14·4%).

p-(2-*Benziminazolyl*)*methylaminobenzoyl-*L*-glutamic Acid.*—The ethyl ester (1 g.) was dissolved in ethanol (10 c.c.) to which a solution of sodium hydroxide (0·5 g.) in water (3 c.c.) was added. After 2 hours the solution was partly evaporated under reduced pressure and treated with a little water containing concentrated hydrochloric acid (1·6 c.c.). The oily precipitate (0·8 g., 90%) shortly solidified, and when crystallised from 2N-hydrochloric acid gave the *hydrochloride* of the substituted glutamic acid in the form of minute prisms, m. p. 201° (Found: C, 52·9; H, 5·0; Cl, 7·55. $C_{20}H_{20}O_5N_4$,HCl,H_2O requires C, 53·3; H, 5·1; Cl, 7·9%. Found, after drying at 100° in a vacuum: C, 55·6; H, 4·7; N, 12·6; Cl, 8·1. $C_{20}H_{20}O_5N_4$,HCl requires C, 55·5; H, 4·9; N, 12·9; Cl, 8·2%).

5-*Chloro*-2-*chloromethylbenziminazole.*—4-Chloro-*o*-phenylenediamine dihydrochloride (5·4 g., 1 mol.) and chloroacetic acid (3·6 g., 1½ mols.) were dissolved in 3N-hydrochloric acid (37 c.c.) which was then heated under reflux for ¼ hour. After being left overnight and treatment with charcoal, the solution was diluted with water (50 c.c.) and basified with 6N-ammonia. The buff precipitate (5·3 g.) was collected, dried, and extracted with cold acetone, which left a residue (3—3·5 g.) probably a condensate of 2 molecules of the benziminazole. The product obtained by evaporating the acetone solution was dissolved in alcoholic hydrogen chloride (charcoal), from which 5-*chloro*-2-*chloromethylbenziminazole hydrochloride* was precipitated by ether as light pink microscopic prisms (0·8 g.), m. p. 213—214° (decomp.) (Found: C, 37·9; H, 3·4. $C_8H_6N_2Cl_2$,HCl,H_2O requires C, 37·6; H, 3·5%). The *base* was precipitated from an aqueous solution of the salt with ammonia as a colourless solid difficult to recrystallise, m. p. after drying over phosphoric anhydride, 140° (Found: C, 47·8; H, 3·1; N, 13·3; Cl, 34·6. $C_8H_6N_2Cl_2$ requires C, 47·8; H, 3·0; N, 13·9; Cl, 35·3%). The *picrate* was prepared from the hydrochloride and picric acid in ethanol and crystallised in yellow elongated prisms, m. p. 195—196° (Found: N, 16·1; Cl, 16·4. $C_8H_6N_2Cl_2,C_6H_3O_7N_3$ requires N, 16·3; Cl, 16·5%).

p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoic Acid.*—The unpurified 5-chloro-2-chloromethylbenziminazole (2 g.), *p*-aminobenzoic acid (1·4 g.), and sodium iodide (0·1 g.) were treated with hot alcohol (25 c.c.), and after filtration the solution was heated under reflux. Colourless solid began to separate in 4 hours, and after 18-hours' heating the product (1·1 g., 37%) was collected, washed with cold ethanol, and dissolved in boiling water (60—70 c.c.) containing a little hydrochloric acid. When kept, the *hydrochloride* crystallised in prisms, m. p. 276° (decomp.) (Found: C, 52·9; H, 4·0; N, 12·3; Cl, 21·4. $C_{15}H_{12}O_3N_3Cl$,HCl requires C, 53·3; H, 3·85; N, 12·4; Cl, 21·0%). Addition of sodium acetate to its solution in water gave the *amino*-acid, crystallising as an alcoholate from aqueous ethanol in tiny plates, sintering at *ca.* 150°, m. p. 237° (decomp.) (Found: C, 58·8; H, 5·1; Cl, 10·4. $C_{15}H_{12}O_3N_3Cl,C_2H_6O$ requires C, 58·7; H, 5·2; Cl, 10·2%). The *picrate*, yellow needles from alcohol, had m. p. *ca.* 235° (decomp.) (Found: C, 47·4; H, 2·6; Cl, 6·9. $C_{15}H_{12}O_3N_3Cl,C_6H_3O_7N_3$ requires C, 47·5; H, 2·8; Cl, 6·7%).

Ethyl p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoylglutamate.*—The *p*-aminobenzoylglutamic ester (1·85 g.) and 5-chloro-2-chloromethylbenziminazole (1·15 g.) gave the required product after boiling in alcohol (10 c.c.) with sodium iodide (0·1 g.) for 15 hours. It was isolated by concentrating the solution (charcoal) and leaving it in the cold, the *hydrochloride* (0·85 g., 30%) separating from ethanol as colourless minute prisms, m. p. 219—220° (Found: C, 54·9; H, 5·2; N, 11·2; Cl, 14·0. $C_{24}H_{27}O_5N_4Cl$,HCl requires C, 55·1; H, 5·35; N, 10·7; Cl, 13·6%). The *amino*-ester crystallised from 50% aqueous alcohol in clusters of colourless needles, m. p. 123° (Found: Cl, 7·4. $C_{24}H_{27}O_5N_4Cl$ requires Cl, 7·2%); it formed a *picrate*, sparingly soluble in alcohol, m. p. 195° (Found: C, 50·6; H, 4·2; N, 13·5; Cl, 5·2. $C_{24}H_{27}O_5N_4Cl,C_6H_3O_7N_3$ requires C, 50·3; H, 4·2; N, 13·7; Cl, 5·0%).

p-(5-*Chloro*-2-*benziminazolyl*)*methylaminobenzoylglutamic Acid.*—The ester (0·3 g.) was dissolved in ethanol (3 c.c.), and a solution of sodium hydroxide (0·15 g.) in water (1 c.c.) added. After 2 hours the mixture was concentrated in a vacuum at room temperature and acidified with 12% hydrochloric acid (1·5 c.c.). The resultant gummy precipitate hardened on rubbing, and the amino-acid *hydrochloride* (0·17 g., 62%) obtained on crystallisation from dilute hydrochloric acid had m. p. 199—202° (Found: C, 49·7; H, 4·6; N, 11·5; Cl, 14·3. $C_{20}H_{19}O_5N_4Cl$,HCl,H_2O requires C, 49·5; H, 4·5; N, 11·5; Cl, 14·6%. Found, after drying at 140°: C, 51·7; H, 4·5; loss, 3·6. $C_{20}H_{19}O_5N_4Cl$,HCl requires C, 51·4; H, 4·3; loss 3·7%).

p-*Nitrobenzoyl-*DL*-methionine.*—*p*-Nitrobenzoyl chloride (10 g.) in dioxan (30 c.c.) and aqueous sodium hydroxide (2·2 g. in 50 c.c.) were added simultaneously to a stirred solution of DL-methionine (8 g.) in a further equal quantity of alkali at room temperature in the course of 25—30 minutes. Stirring was continued until the red-purple solution was almost colourless, and concentrated hydrochloric acid (7—8 c.c.) was then added. The precipitated p-*nitrobenzoyl-*DL*-methionine* had m. p. 145—160° after crystallisation from aqueous alcohol, being contaminated with *p*-nitrobenzoic acid. This was removed

by trituration with ether, in which it is less soluble; crystallisation from hot water containing ethanol then gave the methionine derivative in colourless long thin prisms, m. p. 169—170° (Found: C, 48·4; H, 4·8; N, 9·35; S, 10·6. $C_{13}H_{14}O_5N_2S$ requires C, 48·3; H, 4·7; N, 9·40; S, 10·7%).

p-*Aminobenzoyl*-DL-*methionine*.—*p*-Nitrobenzoylmethionine (3 g.) was dissolved in water (25 c.c.) containing a little ammonia, and a solution of ferrous sulphate heptahydrate (20 g.) in boiling water (40 c.c.) was added. Further additions of aqueous ammonia (total, 40—45 c.c.; *d* 0·88) were made to the boiling liquid until alkaline, and after further brief heating it was filtered and the filtrate and washings concentrated. Neutralisation with acetic acid precipitated the p-*aminobenzoyl*-DL-*methionine* in long colourless needles, m. p. 169—171°, rising when recrystallised to 172° (Found: C, 54·1; H, 6·15. $C_{13}H_{16}O_3N_2S$ requires C, 53·7; H, 6·0%).

p-(2-*Benziminazolyl*)*methylaminobenzoyl*-DL-*methionine*.—A solution of *p*-aminobenzoyl-DL-methionine (1·2 g.), 2-chloromethylbenziminazole (0·8 g.), and sodium iodide (0·1 g.) in ethanol (13 c.c.) was heated under reflux for 5 hours. Ether was added, precipitating a gum which was purified by dissolving it in very dilute hydrochloric acid and reprecipitating with sodium hydrogen carbonate. Several crystallisations from alcohol gave the *benziminazolylmethylaminobenzoylmethionine* in buff globules, m. p. *ca.* 195° (Found: C, 59·3; H, 5·7; N, 14·5. $C_{20}H_{22}O_3N_4S$ requires C, 60·3; H, 5·5; N, 14·1%).

4-*Chloro*-2-*nitrochloroacetanilide*.—Chloroacetyl chloride (22·8 g., 1·1 mols.) was carefully mixed with 4-chloro-2-nitroaniline (28 g., 1 mol.), and after the initial reaction, the mixture was heated until molten. The product was then cooled, ground with water, and dissolved in boiling alcohol. On cooling, the *anilide* (38 g., 79%), m. p. 139°, separated, and two further crystallisations gave long thin pale yellow prisms, m. p. 141° (Found: C, 38·5; H, 2·5; N, 11·7. $C_8H_5O_3N_2Cl_2$ requires C, 38·6; H, 2·4; N, 11·2%).

Anilinoaceto-4-*chloro*-2-*nitroanilide*.—The chloroacetanilide (2·3 g., 1 mol.) and aniline (1·8 g., 2·1 mols.) were heated in propanol (14 c.c.) under reflux for 3 hours, and the product precipitated by water. The *anilinoacetanilide* (2 g., 71%), m. p. 132—133°, after recrystallisation from water formed orange-yellow plates, m. p. 134—135° (Found: C, 55·2; H, 4·0; Cl, 11·8. $C_{14}H_{12}O_3N_3Cl$ requires C, 55·0; H, 3·9; Cl, 11·6%).

When the chloroacetanilide (1·2 g.) and aniline (0·45 g., 1 mol.) were heated in pyridine (3 c.c.), a solid began to separate, and this was redissolved by the addition of more pyridine, which was then refluxed for ½ hour. Next day, the anilinoaceto-4-chloro-2-nitroanilide (0·12 g.) was obtained by filtering the pyridine solution from a crystalline solid and adding water. The crystalline residue was water-soluble and consisted of 4-*chloro*-2-*nitroanilinoacetylpyridinium chloride* (1 g., 61%), which separated from ethanol in pale yellow needles, m. p. 232° (decomp.) (Found: C, 47·4; H, 3·3; N, 13·1. $C_{13}H_{11}O_3N_3Cl_2$ requires C, 47·6; H, 3·4; N, 12·8%).

N-(5-*Chloro*-2-*benziminazolyl*)*methylaniline*.—Anilinoaceto-4-chloro-2-nitroanilide, dissolved in methanol, was reduced over Raney nickel at room temperature under 2 atm. hydrogen pressure. The corresponding 2-*amino*-*anilide* separated from aqueous alcohol in colourless plates, m. p. 163° (Found: Cl, 12·5. $C_{14}H_{14}ON_3Cl$ requires Cl, 12·9%). The amine was warmed with aqueous methanolic hydrogen chloride, and the resulting benziminazole isolated as an unstable *dihydrochloride* in colourless prisms, m. p. 250—251° (Found: C, 51·4; H, 4·8. $C_{14}H_{12}N_3Cl,2HCl$ requires C, 50·8; H, 4·2%). The *picrate*, yellow prisms from aqueous alcohol, had m. p. 214—215° (decomp.) (Found: C, 49·4; H, 3·2; N, 17·6. $C_{14}H_{12}N_3Cl,C_6H_3O_7N_3$ requires C, 49·3; H, 3·1; N, 17·3%).

p-*Carbethoxyanilinoaceto*-2-*nitroanilide*.—2-Nitrochloroacetanilide (4·3 g., 1 mol.), ethyl *p*-aminobenzoate (3·3 g., 1 mol.), and pyridine (1·6 c.c.) were heated in refluxing *n*-propanol (10 c.c.) for 30 hours. Pouring into water gave p-*carbethoxyanilinoaceto*-2-*nitroanilide* which, after crystallisation from ethanol and then *n*-propanol, formed irregular yellow plates (0·82 g., 12%), m. p. 186° (Found: C, 59·4; H, 5·0; N, 12·0. $C_{17}H_{17}O_5N_3$ requires C, 59·5; H, 5·0; N, 12·2%). The omission of pyridine or heating for a shorter time resulted in a still smaller yield.

Hydrogenation of the 2-nitroanilide in methanol at room temperature and 2 atm. gave the corresponding 2-*amino*-compound, which crystallised from alcohol in clusters of colourless needles, m. p. 169° (Found: C, 64·9; H, 6·4; N, 13·0. $C_{17}H_{19}O_3N_3$ requires C, 65·2; H, 6·1; N, 13·4%). Boiling the base in 95% alcoholic hydrogen chloride and evaporation gave the hydrochloride of ethyl *p*-(2-benziminazolyl)methylaminobenzoate, which crystallised from alcohol in prisms, m. p. 255° (decomp.) alone or mixed with the product prepared from 2-chloromethylbenziminazole. The benziminazole picrate, m. p. and mixed m. p. 210°, was obtained by boiling *p*-carbethoxyanilinoacet-2-aminoanilide in saturated ethanolic picric acid (2 mols.) for 4 hours (Found: C, 53·1; H, 3·9; N, 16·1%).

Methyl p-*Nitrobenzoyl*-L-*glutamate*.—Methyl L-glutamate was prepared by heating the acid (3 g.) under reflux with 3% methyl-alcoholic hydrogen chloride. The glassy residue of ester hydrochloride obtained after evaporating the alcohol and triturating with benzene was heated under reflux in benzene (25 c.c.) with *p*-nitrobenzoyl chloride (4 g.) for 7 hours. When cold, the solution was twice washed with aqueous bicarbonate, dried, and evaporated. The residue of *methyl* p-*nitrobenzoyl*-L-*glutamate* (3·1 g., 47%) crystallised from aqueous methanol in hair-fine needles, m. p. 96—97° (Found: C, 51·8; H, 4·9; N, 8·4. $C_{14}H_{16}O_7N_2$ requires C, 51·8; H, 4·9; N, 8·6%).

2-(2′-*Chloroethyl*)*benziminazole*.—A solution of *o*-phenylenediamine (5·4 g., 1 mol.) and β-chloropropionic acid (8·1 g., 1·5 mols.) in 4N-hydrochloric acid (50 c.c.) was boiled for ¼ hour. The solution was then filtered, diluted with water (100 c.c.), cooled to 0°, and neutralised with 6N-ammonia. The nearly colourless precipitate of the benziminazole, rapidly dried over phosphoric anhydride in a vacuum, had m. p. *ca.* 85° (2·5 g., 28%), but it darkened and resinified in a day or two. When the freshly prepared material was dissolved in ethanolic hydrogen chloride (20 c.c.) and the solution treated with ether, a precipitate of 2-(2′-*chloroethyl*)*benziminazole hydrochloride* separated, which, if dissolved in cold acetone–ethanol, could be obtained by the addition of ether as colourless needles, m. p. 180° (Found: C, 49·9; H, 4·7; N, 12·9; Cl, 32·6. $C_9H_9N_2Cl,HCl$ requires C, 49·8; H, 4·6; N, 12·9; Cl, 32·7%). The benziminazole was also formed on adding 2-chloropropionimino ethyl ether hydrochloride to a solution of *o*-phenylenediamine in dry ethanol, heat being evolved and ammonium chloride precipitated; the product was isolated by adding water and ammonia, and identified with the compound obtained by the previous method.

Biological data on the compound [II; R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = H] have already been published (King, Spensley, and Nimmo-Smith, *Nature*, 1948, **162**, 153) and show that it has a low order of inhibitory activity for *S. fæcalis* R. and *L. casei*. The inhibitory power of the 5-chloro-analogue [II, R = NH·CH(CO_2H)·CH_2·CH_2·CO_2H, X = Cl] for *L. casei* is somewhat more marked; its bacterial index is 10^6. The simpler acid (II; R = OH, X = Cl) is inactive for *L. casei*, as is the methionine derivative [II, R = NH·CH(CO_2H)·CH_2·CH_2·SMe, X = H] for *S. fæcalis* R.

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Dysons Perrins Laboratory, Oxford University. [*Received, November 10th*, 1948.]

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299. *Deoxypentose Nucleic Acids. Part IV. The Electrophoresis of the Deoxypentose Nucleic Acid of Calf Thymus.*

By J. M. Creeth, D. O. Jordan, and (the late) J. Masson Gulland.

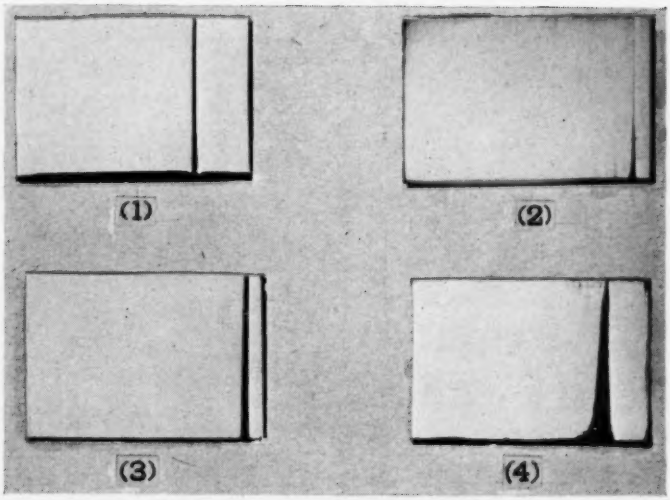
The electrophoresis of the sample of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus prepared by Gulland, Jordan, and Threlfall (*J.*, 1947, 1129) has been studied in the Tiselius apparatus. The variation of the mobility with changes in concentration, ionic strength, and pH have been investigated. The results, which are in agreement with and extend those of previous workers, are interpreted in terms of the macromolecular structure of this nucleic acid (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131; Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141).

The results of previous investigations of the electrophoretic mobility of deoxypentose nucleic acids (Stenhagen and Teorell, *Trans. Faraday Soc.*, 1939, **35**, 743; Seibert, *J. Biol. Chem.*, 1940, **133**, 593; Hall, *J. Amer. Chem. Soc.*, 1941, **63**, 794; Zittle and Seibert, *J. Immunol.*, 1942, **43**, 47; Cohen, *J. Biol. Chem.*, 1942, **146**, 471) have shown that the deoxypentose nucleic acid of calf thymus is electrophoretically homogeneous with a high mobility towards the anode. The various recorded values of the mobility, however, show some discrepancy which can be attributed only in part to the different ionic strengths employed in these investigations. These variations have been ascribed by Cohen (*loc. cit.*) to a difference in the number of phosphoric acid dissociations per molecule in the various samples studied; furthermore it has been demonstrated that the molecular weight is dependent on the method of extraction (Schmidt, Pickels, and Levene, *J. Biol. Chem.*, 1939, **127**, 251; Tennent and Vilbrandt, *J. Amer. Chem. Soc.*, 1943, **65**, 424), and that treatment with acid or alkali produces irreversible changes in the macromolecular structure (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131; Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141). It was therefore considered desirable that the sample of the sodium salt of the deoxypentose nucleic acid of calf thymus which had been prepared and analytically characterised by Gulland, Jordan, and Threlfall (Part I, *J.*, 1947, 1129) and shown by electrometric titration (Gulland, Jordan, and Taylor, *loc. cit.*) and viscosity measurements (Creeth, Gulland, and Jordan, *loc. cit.*) to be little, if at all, degraded, should be subjected to an electrophoretic study.

Results of the Present Investigation.—In conformity with the results of previous investigators, this sample of deoxypentose nucleic acid was found to be electrophoretically homogeneous under all the conditions of ionic strength and pH studied (Fig. 1). In agreement with expectation from the known high molecular weight and monodisperse character of this material (Cecil and Ogston, *J.*, 1948, 1382), diffusion occurred slowly, and the boundaries remained sharp during the course of electrophoresis.

(i) *The variation of mobility with concentration of deoxypentose nucleic acid.* Stenhagen and Teorell (*loc. cit.*) observed that the mobility of a sample of the sodium salt of deoxypentose nucleic acid prepared by the method of Bang (Hofmeister's "Beiträge chem. Physiol. Path.," 1903, **4**, 331) as modified by Hammarsten (*Biochem. Z.*, 1924, **144**, 383) was independent of the concentration over the concentration range 0·05—0·25%, although the viscosity of the solutions was more than doubled over this range. Our results, which were obtained at constant ionic strength (0·20 due entirely to NaCl) are given in the table, and show only a very slight increase of mobility with concentration although the viscosity has increased by more than tenfold. This result thus confirms the conclusion of Stenhagen and Teorell (*loc. cit.*) that a viscosity correction should not be applied to these measurements.

FIG. 1.



Electrophoretic patterns of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus. Concentration 0·2%, *ionic strength* 0·2. *The ascending boundary (migration towards the anode) is shown in each case:* (1) *after* 12×10^3 *secs. at* 1·42 *volt/cm., phosphate–chloride buffer, pH* 7·0; (2) *after* $6{\cdot}12 \times 10^3$ *secs. at* 2·16 *volt/cm., phosphate buffer, pH* 11·7; (3) *after* $10{\cdot}38 \times 10^3$ *secs. at* 2·90 *volt/cm., acetate–chloride buffer, pH* 3·57; (4) *after* $13{\cdot}92 \times 10^3$ *secs. at* 1·57 *volt/cm., phosphate–chloride buffer, pH* 6·70, *after alkaline treatment at pH* 12·0.

[*To face p.* 1406.

Concn. of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus (%).	Electrophoretic mobility towards anode.[1]	Relative viscosity [2] at an applied pressure of 8000 dynes/cm.[2].
0·05	1·39	3
0·10	1·44	7
0·20	1·43	21
0·30	1·50	40

[1] These and all subsequent values of mobilities are stated in μ/sec./(volt/cm.).
[2] Values of the viscosity were determined by the method of Creeth, Gulland, and Jordan (*loc. cit.*).

(ii) *Dependence of the mobility on the ionic strength.* The ionic strength was varied by using sodium chloride as added electrolyte. The absence of buffering capacity of the deoxypentose nucleate ion at pH 7·0 (Gulland, Jordan, and Taylor, *loc. cit.*) renders the mobility change across the boundary negligible, and the migration is free from boundary anomalies. The results obtained are shown in Fig. 2, and it is evident that the mobility is greatly influenced by the ionic strength. There is a close similarity between the variation of mobility and the variation of viscosity with ionic strength (Creeth, Gulland, and Jordan, *loc. cit.*) which is in accordance with the suggestion that the change of viscosity with ionic strength is due to changes in the structure of the ionic atmosphere (Gulland and Jordan, *Symp. Soc. Exp. Biol.*, 1947, **1**, 56). The interpretation of these data with reference to the theory of electrophoretic migration is discussed in Part V (following paper).

FIG. 2.

Variation of the electrophoretic mobility of the deoxypentose nucleate ion with ionic strength in solutions of sodium chloride.

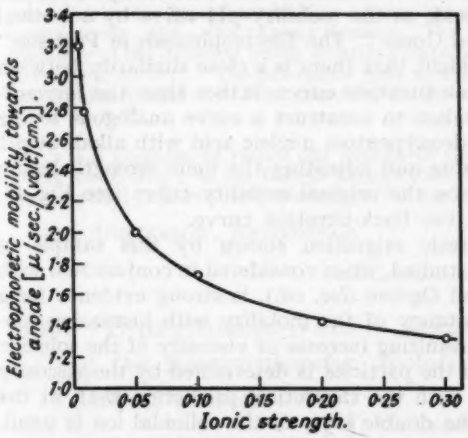
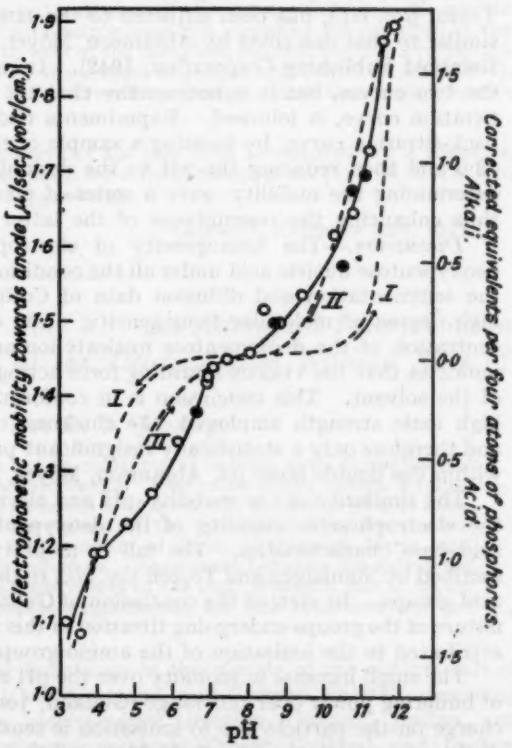


FIG. 3.

Variation of the electrophoretic mobility of the deoxypentose nucleate ion with pH. Ionic strength 0·20.



○ *Solutions brought directly to the given pH;* ● *solutions brought to the given pH after treatment with alkali at pH 12·0. Broken curve I, forward titration with acid or alkali from pH 7·0; broken curve II, backward titration with acid from pH 12·0 or with alkali from pH 2·5.*

(iii) *The variation of mobility with pH.* Stenhagen and Teorell (*loc. cit.*) investigated the acid–base binding and mobility–pH curves for the sample of deoxypentose nucleic acid studied by them, and reported a general similarity between the two. Their measurements, however, were restricted to the pH range 2·5—10·0, and, in view of the change in macromolecular structure occurring at pH values greater than 10·0 and the subsequent titration of the purine-pyrimidine hydroxyl groups between pH 10·0 and 12·0, it was expected that the mobility changes over this pH range would prove of interest; measurements of the mobility have therefore been made over the range pH 3·0—12·0.

The buffer solutions used were acetate, phosphate, borate, veronal, citrate, and carbonate, and in general the buffer contributed to not more than half the final ionic strength (0·20), the remainder being sodium chloride. By this means and by avoiding the use of potassium salts (Longsworth and MacInnes, *Chem. Reviews*, **1939**, **24**, 271; Longsworth, *Ann. N. Y. Acad. Sci.*, **1941**, **41**, 267) specific effects due to individual buffer ions were minimised. However, citrate buffers were found to give abnormally high mobilities of the deoxypentose nucleate ion, thus resembling the behaviour of phycoerythrin in these buffers reported by Tiselius (*Nova Acta Reg. Soc. Sci. Upsaliensis*, **1930**, **1**, No. 4), and borate buffers were found to give rather low mobilities in agreement with the observations of Stenhagen and Teorell (*loc. cit.*). In the latter case the anomalous behaviour disappeared as the borate concentration was reduced at constant ionic strength. The mobility values in veronal buffers, which are recommended by Longsworth, Shedlovsky, and MacInnes (*J. Exp. Med.*, **1939**, **70**, 399) for electrophoresis measurements, were almost identical with those obtained in phosphate buffers.

A representative series of photographs of the boundaries after electrophoresis under various conditions is shown in Fig. 1, and the mobility–pH curve obtained, together with the acid–base binding curve, is given in Fig. 3. The latter curve, taken from the data of Gulland, Jordan, and Taylor (*loc. cit.*), has been adjusted to the same scale as the mobility–pH curve by a method similar to that described by Abramson, Moyer, and Gorin (" The Electrophoresis of Proteins," Reinhold Publishing Corporation, 1942). It is evident that there is a close similarity between the two curves, but it is noteworthy that the back-titration curve, rather than the forward-titration curve, is followed. Experiments undertaken to construct a curve analogous to the back-titration curve, by treating a sample of the deoxypentose nucleic acid with alkali at pH 12·0 and then reducing the pH to the desired value and adjusting the ionic strength before determining the mobility, gave a series of points on the original mobility curve (see Fig. 3), thus enhancing the resemblance of the latter to the back-titration curve.

Discussion.—The homogeneity of electrophoretic migration shown by this sample of deoxypentose nucleic acid under all the conditions studied, when considered in conjunction with the sedimentation and diffusion data of Cecil and Ogston (*loc. cit.*), is strong evidence for a high degree of molecular homogeneity. The constancy of the mobility with increasing concentration of the deoxypentose nucleate ion and resulting increase of viscosity of the solution indicates that the viscous retarding force acting on the particles is determined by the viscosity of the solvent. This conclusion is in conformity with the theoretical prediction that, at the high ionic strength employed, the thickness of the double layer of the colloidal ion is small, and therefore only a statistically insignificant proportion of the colloidal solute can be included within the double layer (cf. Abramson, Moyer, and Gorin, *loc. cit.*).

The similarity of the mobility–pH and electrometric titration curves (Fig. 3) indicates that the electrophoretic mobility of the deoxypentose nucleate ion is largely dependent on the acid–base characteristics. The fall in mobility as the pH is decreased from neutrality was ascribed by Stenhagen and Teorell (*loc. cit.*) to the decreased ionisation of the primary phosphoric acid groups. In view of the conclusion of Gulland, Jordan, and Taylor (*loc. cit.*) concerning the nature of the groups undergoing titration in this range this view is no longer tenable, and it is now attributed to the ionisation of the amino-groups causing a decrease in the net negative charge.

The small increase in mobility over the pH range 7·0—10·0 is in agreement with the absence of buffering power over this range (Gulland, Jordan, and Taylor, *loc. cit.*) when that part of the charge on the particle due to ionisation is sensibly constant. The sharp increase in the slope of the curve in the region of pH 11·0, and the maximum value of the mobility attained at pH 12·0, are consistent with the view, suggested by the electrometric titration and viscosity data, that there is a sudden liberation of dissociating groups from an initially hydrogen bonded aggregate.

In view of the absence of any difference between the mobilities of the normal and alkali-treated samples of this substance in the region of pH 10·0, similar to the discrepancy in this region of the forward- and back-titration curves (Gulland, Jordan, and Taylor, *loc. cit.*), it must be concluded that the factors which determine the mobility of the less asymmetric particles considered to be produced by this change are similar to those previously operative on the original aggregates. The further interpretation of the mobility data in this pH region and also at pH 5 must await a more complete knowledge of the size, shape, and charge of the deoxypentose nucleate ion.

Experimental.

Solutions were prepared by dissolving in water, with stirring, a known weight of the sodium salt of deoxypentose nucleic acid, previously dried in a vacuum at 100° over phosphoric oxide, to give a

solution of twice the desired concentration. This solution was then diluted with an equal volume of buffer solution of twice the desired ionic strength. By adopting this procedure, the need for prolonged dialysis was avoided. This was considered necessary in view of the changes in macromolecular structure which result from prolonged contact with acid or alkaline solutions.

The mobilities were determined at 0·5° in a modified form of the electrophoresis apparatus (constructed by Adam Hilger Ltd.) described by Tiselius (*Trans. Faraday Soc.*, 1937, **33**, 524) and equipped with the cylindrical lens and diagonal edge form of optical system introduced by Philpot (*Nature*, 1938, **141**, 283). The conditions necessary for the accurate determination of mobilities with this apparatus have been described by Tiselius (*loc. cit.*), Longsworth and MacInnes (*J. Amer. Chem. Soc.*, 1940, **62**, 705), and Svensson (*Arkiv Kemi, Min. Geol.*, 1946, **22**, *A*, No. 10). A colloid concentration of not more than 0·20% was used, and where possible buffer solutions of high ionic strength were employed. Under these conditions the specific conductivities of the deoxypentose nucleic acid solutions and of the buffer solutions were sensibly identical, and boundary anomalies almost completely depressed.

The movements of the boundaries were recorded photographically, four positions of the boundary generally being obtained during an experiment over a period of 3—4 hours. During this time the movement of the boundary was of the order of 4 cm. Measurement of the distances on the photographic plate was made with a travelling microscope reading to 0·002 cm. The motions of both the ascending and descending boundaries were in general identical within the limits of error of measurement, and mobilities were therefore generally calculated from the ascending boundaries which were somewhat sharper. Mobilities were calculated from the formula $u = \Delta x.q.k_s/i.\Delta t$ where Δx is the distance travelled by the boundary in time Δt, q is the cross-sectional area of the U-tube limb, k_s is the specific conductivity, and i the current flowing. The mobility values were not corrected for variation of the viscosity of the different buffer solutions employed in view of the uncertain significance of this correction (Svensson, *loc. cit.*). The pH values were measured by the method of Jordan and Taylor (*J.*, 1946, 994), and conductivities were determined by the use of a Mullard resistance bridge. Both measurements were made at 0·5°.

It is a pleasure to record our thanks to the Trustees of the late Lord Leverhulme for a Research grant (to D. O. J.), to the British Empire Cancer Campaign for a Maintenance grant (to J. M. C.), and to the Imperial Chemical Industries Ltd. for the loan of apparatus.

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300. *Deoxypentose Nucleic Acids. Part V. An Attempted Interpretation of the Electrophoretic Mobilities of the Deoxypentose Nucleic Acid of Calf Thymus.*

By J. M. CREETH and D. O. JORDAN.

The charge on the deoxypentose nucleate ion in solutions of various ionic strengths has been determined from membrane potential measurements. The value of the charge is found to be considerably lower than that calculated from the analytical and molecular-weight data, and the reason for this is discussed. From the values of the particle charge, the electrophoretic mobility has been calculated from the equation of Gorin (Abramson, Gorin, and Moyer, *Chem. Reviews*, 1939, **24**, 345) for long cylinders. Agreement between the calculated and observed values is found to be excellent at low values of the ionic strength, but at higher values considerable discrepancies occur; possible explanations for this behaviour are put forward.

DURING an investigation of the electrophoretic behaviour of the sample of the sodium salt of the deoxypentose nucleic acid of calf thymus (Creeth, Jordan, and Gulland, Part IV, preceding paper) which had previously been studied by electrometric titration (Gulland, Jordan, and Taylor, Part II, *J.*, 1947, 1131) and by viscosimetric methods (Creeth, Gulland, and Jordan, Part III, *J.*, 1947, 1141), a large variation of the mobility of the deoxypentose nucleate ion with change of ionic strength was observed. The molecules of this nucleic acid have been shown to be cylindrical in shape with a large axial ratio (see, *e.g.*, Signer, Caspersson, and Hammarsten, *Nature*, 1938, **141**, 122; Astbury, *Symp. Soc. Exp. Biol.*, 1946, **1**, 66; Cecil and Ogston, *J.*, 1948, 1382), and furthermore it has been shown that the particular sample of this nucleic acid referred to above is homogeneous as to molecular size and weight (Cecil and Ogston, *loc. cit.*). It was therefore considered that the electrophoretic data obtained by Creeth, Jordan, and Gulland (*loc. cit.*) presented an opportunity of testing the present electrokinetic theory of long cylindrical particles, and possibly of gaining further light on the unusual physical properties of solutions of the sodium salt of the deoxypentose nucleic acid of calf thymus, *e.g.*, the very high viscosity.

Few attempts have previously been made to interpret the observed mobility characteristics of biocolloids. The work of Tiselius and Svensson (*Trans. Faraday Soc.*, 1940, **36**, 16) on egg albumin, which demonstrated a close correspondence between the observed and cal-

culated mobilities over a range of ionic strength, has been criticised by Gorin (*J. Physical Chem.*, 1941, **45**, 371), who considered the refinements to the Henry–Debye–Hückel theory which should be applied. Adair and Adair (*Trans. Faraday Soc.*, 1940, **36**, 23) also obtained satisfactory agreement between the observed and calculated mobilities of hæmoglobin by the use of the uncorrected Henry–Debye–Hückel equation. In both these cases the protein molecules were assumed to be spherical. Gorin and Moyer (*J. Gen. Physiol.*, 1942, **25**, 785) have applied a theoretical equation for the mobilities of cylindrical particles to the data for serum albumin-*B*. Good agreement was obtained between the particle charge values calculated from mobilities and those calculated from electrometric titration data over a small range of pH close to the isoelectric point. The asymmetry of the molecules of this protein is, however, small.

The most satisfactory method of testing the validity of electrokinetic equations is to obtain values of the particle charge by an independent means and then to calculate theoretical mobilities. Such particle charge values may, in certain cases, be determined from the acid–base binding curve of the substance; the problem is complicated, however, by the binding of ions other than hydrogen or hydoxyl, and Abramson, Moyer, and Gorin ("The Electrophoresis of Proteins," Reinhold Publishing Corporation, 1942) have concluded that, for proteins, accurate values of the particle charge can only be obtained from titration measurements which have been extrapolated to zero ionic strength and zero protein concentration. This method clearly cannot give values of the particle charge over a range of ionic strengths, and we have therefore employed the method described by Tiselius and Svensson (*loc. cit.*) and Adair and Adair (*loc. cit.*) in which the particle-charge values are obtained from membrane potential measurements. The membrane potential is the potential existing between a colloidal solution and a buffer solution from which it is separated by a membrane, permeable to the electrolyte ions but impermeable to those of the colloid, the two solutions being in Donnan equilibrium. This method, initiated by Loeb ("Proteins and the Theory of Colloidal Behaviour," New York, 1922) and developed by Adair and Adair (*Biochem. J.*, 1934, **28**, 199; *loc. cit.*), gives values for the equivalent concentration of the colloidal ion, and thus, if the molecular weight is known, the particle charge can be determined. By this method it is possible to obtain values of the particle charge under conditions of different ionic strength.

Results.—The results of the determination of the particle charge of the deoxypentose nucleate ion are shown in Table I. The values of the equivalent concentration (c) of the deoxypentose nucleate ion were calculated from the equation

$$c = [\text{sum of equivalents of cations}]_2 - [\text{sum of equivalents of anions}]_2$$

where the terms within the brackets and designated by the subscript 2 refer to the concentrations of the solution within the membrane containing the colloidal ion. These concentrations are related to those outside the membrane (designated by the subscript 1) by the equations

$$[\text{cations}]_2 = f[\text{cations}]_1$$

and

$$[\text{anions}]_2 = [\text{anions}]_1/f$$

where f is the ideal distribution ratio for univalent ions and is related to the membrane potential (E) by the equation

$$E = -\frac{RT}{F}\ln f$$

The above ideal equations may only be applied where (i) the liquid junction potentials, KCl/solution 1 and KCl/solution 2 are equal and (ii) the activity coefficients of the ions on both sides of the membrane are equal. It is general to assume (i) to be correct, and Adair and Adair (1934, *loc. cit.*) have shown that if the membrane potential is small (ii) may also be assumed without serious error.

The values of the particle charge were calculated from the equivalent concentration data, the value $8{\cdot}2 \times 10^5$ for the molecular weight of the deoxypentose nucleate ion (Cecil and Ogston, *loc. cit.*) being used.

The values given in Table I, which have been obtained by the approximate elimination of liquid junction potentials by means of concentrated potassium chloride, must be regarded as conventional (Adair and Adair, *loc. cit.*). Moreover in view of the small magnitude of most of the membrane potentials and the errors inherent in the method, the results, particularly at the higher ionic strengths, can only be regarded as approximate. The great interest, however, attached to even approximate data renders the method of value.

TABLE I.

The charge on the deoxypentose nucleate ion at pH 7.

Concn. of deoxypentose nucleate ion (%).	Composition of buffer.	Ionic strength (I).	Membrane potential (E) (mv.).	$c \times 10^8$.	Charge (xe^{-1}).
0·8	NaCl	0·20	0·63	10·70	1090
0·8	NaCl	0·20	0·54	8·10	(830)
0·6	NaCl	0·20	0·54	8·10	1100
0·6	NaCl	0·08	0·95	6·47	882
0·6	Sodium phosphates	0·05	1·19	5·11	698
0·6	NaCl	0·02	2·67	4·50	615
0·6	Sodium phosphates	0·01	4·80	3·70	508
0·6	NaCl	0·005	8·59	3·73	509

Values of the electrophoretic mobility can be calculated by the equation developed by Gorin (see Abramson, Gorin, and Moyer, *Chem. Reviews*, 1939, **24**, 345; Abramson, Moyer, and Gorin, *loc. cit.*) :

$$u = \frac{2Q}{(l + 2a) \cdot F'(\kappa a) \cdot \pi\eta} \cdot \left[\frac{K_0(\kappa a + \kappa r_1)}{(\kappa a + \kappa r_1) \cdot K_1(\kappa a + \kappa r_1)} + \ln\left(\frac{a + r_1}{a}\right)\right]$$

where u is the electrophoretic mobility, Q is the charge on the particle, l and a are respectively the length and the radius of the cylindrical colloid particle, r_1 is the average radius of the ions in the ionic atmosphere, κ is the Debye–Hückel ionic strength function, $F'(\kappa a)$ is the function developed by Gorin to account for the random orientation of the particles, η is the coefficient of viscosity of the solvent and K_0 and K_1 are Bessel functions, values of which together with values of $F'(\kappa a)$ have been given by Abramson, Moyer, and Gorin (*loc. cit.*).

The values of the electrophoretic mobility calculated from this equation are given in Table II, together with the observed values. For these calculations the values of l and a were taken as 2538 A. and 10·6 A. respectively; these values correspond to a value for the axial ratio of 120 and a molecular weight of $8·2 \times 10^5$ (Cecil and Ogston, *loc. cit.*) and a specific volume of 0·55 c.c. per g. The particle charge data used in the calculations were taken from a smooth graph obtained from the data given in Table I. The value of r_1 was taken as 2·24 A., this being the mean of the values for the sodium and chloride ions (Gorin, *J. Chem. Physics*, 1939, **7**, 405). The value of κ at 0·5° is $0·323 \times 10^8\sqrt{I}$, and the viscosity of water at this temperature is $1·76 \times 10^{-2}$ poise.

TABLE II.

Calculated and observed values of the electrophoretic mobility of the deoxypentose nucleate ion at pH 7 in solution of sodium chloride.

Ionic strength (I).	$\sqrt{I}$.	$\kappa(a + r_1)$.	$F'(\kappa a)$.	$\frac{K_0(\kappa a + \kappa r_1)}{K_1(\kappa a + \kappa r_1)}$.	Q.	Mobility, calc.*	Mobility, obs.*
0·005	0·0707	0·293	6·53	0·440	550	3·21	3·20
0·01	0·100	0·414	6·48	0·515	565	2·81	2·79
0·02	0·141	0·584	6·44	0·591	595	2·49	2·48
0·05	0·224	0·913	6·31	0·692	690	2·31	2·02
0·08	0·283	1·17	6·10	0·738	780	2·37	1·83
0·10	0·316	1·31	5·97	0·754	830	2·41	1·74
0·20	0·447	1·85	5·72	0·800	1000	2·46	1·43

* Mobility given in μ/sec./(volt/cm.).

Discussion—(i) *Membrane potentials and particle charge.* The sources of error involved in the determination of membrane potentials have been described in detail by Adair and Adair (1934, *loc. cit.*), and as due consideration was given to their conclusions it is thought to be unlikely that in this investigation the experimental errors were greater than those accounted for by them.

The variation of the particle charge with ionic strength (Table I) is very great and, moreover, at all ionic strengths studied the charge is much lower than the value of 2,664 calculated from the analytical data assuming that the formula of the polynucleotide ion is

$$[(C_{39}H_{43}O_{24}N_{15}P_4)^{4-}]_{666},$$

which corresponds to the molecular weight of $8·2 \times 10^5$, that the primary phosphoric acid groups are completely dissociated, and that the amino-groups are in the uncharged form at pH 7.

This variation of the particle charge from the theoretical value may be compared with the phenomena observed with Congo-red (Donnan and Harris, *J.*, 1911, **99**, 1554; Adair and Adair, 1934, *loc. cit.*) and with gum arabic (Svensson, *Arkiv Kemi, Min. Geol.*, 1946, **22**, *A*, No. 10). In both cases the equivalent concentration of the colloid as determined by membrane-equilibrium measurements was approximately half that calculated on the assumption of complete dissociation. The discrepancy in the case of the deoxypentose nucleate ion is considered to be due to the enmeshing of some of the sodium ions within the colloid micelle, thus reducing the apparent equivalent concentration of the sodium ions on which the membrane potential is dependent. The actual mechanism of this process is most probably ion-pair formation (see Svensson, *loc. cit.*) between sodium ions and phosphoric acid groups of the polynucleotide. Such sodium ions being bound within the surface of shear of the particle will not form part of the outer diffuse layer and will thus reduce the net charge on the particle.

The discrepancy between the observed and calculated values of the particle charge might be considered at first sight to be related to the anomalous osmotic properties of the deoxypentose nucleate ion reported by Hammarsten (*Biochem. Z.*, 1924, **144**, 383), who found that the osmotic pressures of solutions of this substance were lower than the values expected from electrical conductivity measurements. This "Hammarsten effect" was originally ascribed to the enmeshing of some of the sodium ions by the colloidal micelle thus rendering them osmotically inactive. Although it seems certain, from the data reported above, that this process does occur, it cannot explain the discrepancy between the osmotic pressure and conductivity data since the process of ion-pair formation would unavoidably lead to the prevention of such bound sodium ions taking part in the conduction process. The discrepancy between expected and observed osmotic pressures is therefore much more likely to be due to the great deviations from ideal behaviour which must be expected to occur with solutions of such interacting particles as deoxypentose nucleate ions (Linderström-Lang, *Compt. rend. Trav. Lab. Carlsberg*, 1926, **16**, No. 16; van Rysselberghe, *J. Physical Chem.*, 1934, **38**, 645), especially in the absence of a swamping concentration of electrolyte (Hartley, *Quart. Reviews*, 1948, **2**, 152).

The very great increase of particle charge with ionic strength cannot be ascribed solely to a variation in the extent of ion-pair formation, and is probably due to an increase in the adsorption of anions (either chloride or phosphate) by the deoxypentose nucleate ion as the ionic strength increases. This explanation may be compared with that put forward by Adair and Adair (*Biochem. J.*, 1934, **28**, 1230) to account for the behaviour of certain proteins.

(ii) *The calculation of electrophoretic mobility.* It is evident from the results given in Table II that there is close agreement between the observed and calculated mobilities at the lowest ionic strengths studied, but that discrepancies occur as the ionic strength is increased. While these discrepancies may in part be due to errors in the values of the particle charge incurred by the very small magnitude of the membrane potentials in this region, it is necessary to consider other factors that might possibly contribute to them. First, the actual charge effective in determining the mobility may not be identical with that given by membrane measurements. This explanation is considered to be unlikely since an ion which is adsorbed on the micelle sufficiently closely not to form part of the outer diffuse layer when the latter is undisturbed by the presence of an electric field would not normally be expected to become free when the field is applied (Svensson, *loc. cit.*).

Secondly, it is possible that at the higher ionic strengths considered, the charge on the particle is such that there are significant deviations from the assumption, made in the Debye–Hückel theory, that the electrical energy of the ions of the ionic atmosphere is small compared with their thermal energy. It is generally stated that the electrical energy term to be considered is $e\zeta$ (see, *e.g.*, Gorin, *loc. cit.*), where e is the electronic charge and ζ is the electrokinetic potential. However, the Debye–Hückel theory states that, for a sphere, the radial divergence of the potential gradient at any point in the ionic atmosphere is directly proportional to the potential at that point, provided that this potential is such that the electrical energy of an ion *at that point* is small compared with its thermal energy. Thus the energy term $e\zeta$ will be greater than the energy of most ions in the atmosphere, as relatively few will be in contact with the surface of shear, and, moreover, at high ionic strengths the potential falls sharply with the distance from the surface. Thus it is not possible to state any given value of the electrokinetic potential above which the Debye–Hückel theory does not apply, and tests of the Debye–Hückel theory generally consist of a direct comparison of the calculated and experimental values as carried out above. For spherical particles, Gorin (1941, *loc. cit.*; 1942, *loc. cit.*; private communication) has developed the Gronwall, La Mer, and Sandved

(*Physikal. Z.*, 1928, **29**, 358) extension of the Debye–Hückel theory; the resulting expression for the electrophoretic mobility can be used for particles of high charge, and gives mobilities lower in value than those given by the Debye–Hückel theory. A similar expression, applicable to cylindrical particles, has yet to be developed. It is thus concluded that present electrokinetic theory is inadequate to explain the mobility of cylindrical particles in solutions of ionic strength greater than about 0·05.

Experimental.

The determination of electrophoretic mobility has been described by Creeth, Jordan, and Gulland (Part IV, *loc. cit.*).

The membranes for the membrane-potential measurements were made by coating the inside of a boiling tube with two coats of a pyroxylin solution made according to the directions of Adair and Adair (*loc. cit.*), but were somewhat more porous than those described by these authors. In all other respects the apparatus employed was identical with that described by them except that normal calomel electrodes were employed and that in some measurements silver–silver chloride electrodes were used.

The solutions were prepared by dissolving a known weight of the tetrasodium salt of deoxypentose nucleic acid (previously dried in a vacuum at 100° over phosphoric oxide) in the appropriate buffer solution or solution of sodium chloride. The outer solutions, which did not contain the nucleic acid, were replaced by fresh solutions at intervals until further replacement produced no alteration in the membrane potential. The membrane potentials were measured on a potentiometer reading to 70 mv., graduated in steps of 0·02 mv. used in conjunction with a mirror galvanometer having a full scale deflection of 2 mv. All measurements were made at 0·5°.

The procedure for the determination of the membrane potential was as follows. The difference in potential of the two reference electrodes was first measured by placing them in opposition; two measurements of the membrane potential were then made followed by a repeat of the whole procedure with the two electrodes exchanged. The steady reading of the potential obtained 5 minutes after forming the liquid junction was taken as the observed membrane potential (Maclagan, *Biochem. J.*, 1929, **23**, 309).

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301. *Deoxypentose Nucleic Acids. Part VI. Electrometric Titration of the Acidic and Basic Groups of the Deoxypentose Nucleic Acids of Lamb Thymus and Herring Sperm.*

By D. J. Cosgrove and D. O. Jordan.

The acidic and basic groups of the tetrasodium salts of the deoxypentose nucleic acids of lamb thymus and herring sperm have been titrated electrometrically, employing hydrogen electrodes. The conclusions drawn regarding the structure of these nucleic acids are very similar to those arrived at for the deoxypentose nucleic acid of calf thymus (*J.*, 1947, 1131).

In Part II of this series (Gulland, Jordan, and Taylor, *J.*, 1947, 1131) it was shown that the electrometric titration of a sample of the deoxypentose nucleic acid of calf thymus which had been prepared by a method which avoided the use of acid or alkali (Gulland, Jordan, and Threlfall, Part I, *J.*, 1947, 1129) showed anomalous behaviour. On addition of acid or alkali to a solution of the nucleic acid in water no groups were titrated at first between pH 5·0 and 11·0, but outside these limits there occurred a rapid liberation of groups which were titrated in the ranges pH 2·0—6·0 and pH 9·0—12·0. These groups were shown to be the $-NH_2$ and the $-NH-CO-$ groups of the purines and pyrimidines. Back-titration with either acid from pH 12·0 or alkali from pH 2·5 gave a titration curve which was different from that representing the initial titration but identical with the calculated curve. This behaviour was interpreted as showing evidence for the existence of hydrogen bonds between the $-NH_2$ and the $-NH-CO-$ groups of the nucleic acid macromolecule. In view of this interesting conclusion it was considered worth investigating deoxypentose nucleic acids from other sources. The acids chosen for the present investigation were those of lamb thymus and herring sperm.

Results.—The electrometric titration curves, which were corrected at the extremes of pH for the titration of the water by the method of Jordan and Taylor (*J.*, 1946, 994), are shown in Figs. 1 and 2. In both it is seen that the anomalous behaviour observed with the deoxypentose

nucleic acid of calf thymus (Gulland, Jordan, and Taylor, *loc. cit.*), and described above, occurs. The back-titration curves exhibit well-defined points of inflection in the region of pH 7·0, and show incipient points of inflection in the regions pH 11·8 and 2·5, corresponding respectively to 2·0 equivalents of alkali and 3·0 equivalents of acid for each four atoms of phosphorus.

FIG. 1.

The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of lamb thymus.

FIG. 2.

The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of herring sperm.

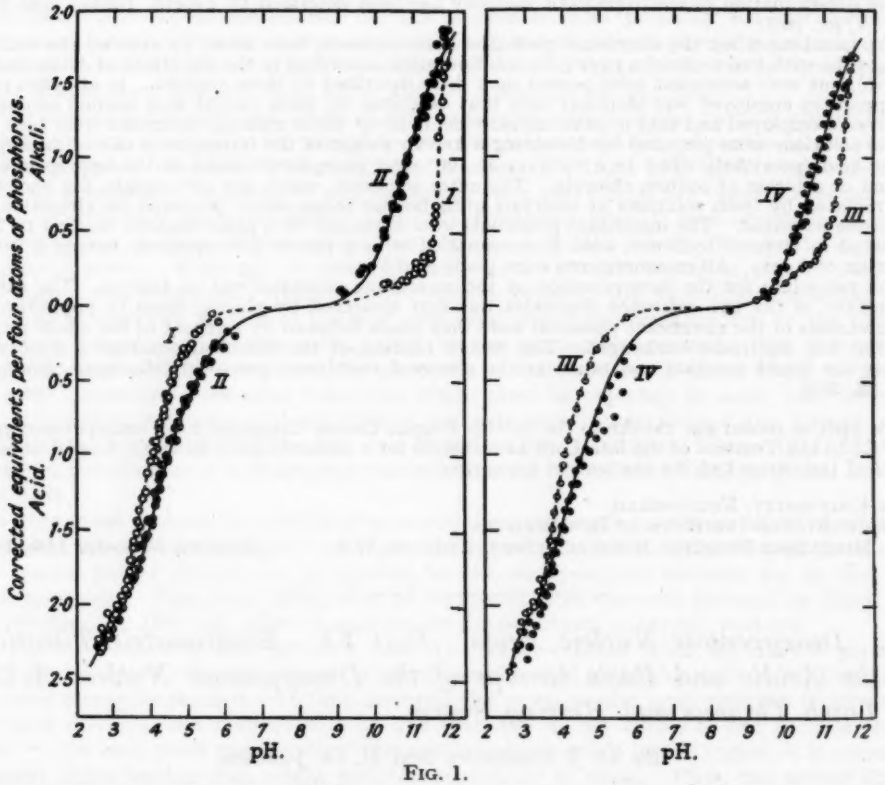

FIG. 1.

I. Titration with acid or alkali from pH 7·0, ○.
II. Back titration with acid from pH 12·0, ◑ and with alkali from pH 2·5, ◐. The smooth curve through these points is calculated for 1·2 equivalents of pK_a' 2·3, 0·8 equivalent of pK_a' 4·1, 1·0 equivalent of pK_a' 4·75, 0·2 equivalent of pK_a' 6·4, and 1·0 equivalent each of pK_a' 10·4 and pK_a' 11·4.

FIG. 2.

III. Titration with acid or alkali from pH 7·0, ○.
IV. Back titration with acid from pH 12·0, ◑ and with alkali from pH 2·5, ◐. The smooth curve through these points is calculated for 1·2 equivalents of pK_a' 2·3, 0·8 equivalent of pK_a' 4·0, 1·0 equivalent of pK_a' 4·8, 0·25 equivalent of pK_a' 6·4, and 1·0 equivalent each of pK_a' 10·4 and pK_a' 11·4.

Discussion.—The nature of the acidic and basic groups undergoing titration in the various pH ranges has been fully discussed in Part II (*loc. cit.*) and, although the same interpretation is made of the results recorded here, *viz.*, that the groups being titrated in the range pH 2·0—5·0 are the $-NH_3^+$ groups and those in the range pH 8·0 to 12·0 are the $-NH-CO-$ groups, it is clear that slight differences in the pK_a' values of the amino-groups exist in the nucleic acids from different sources. Furthermore, the presence of only a small amount of a secondary phosphoric acid dissociation, when considered in conjunction with the sodium analysis of the sodium salt of the deoxypentose nucleic acid of lamb thymus, which shows that there are four atoms of sodium for every four atoms of phosphorus, is consistent with the view that this deoxypentose nucleic acid, like that from calf thymus, has a long unbranched chain structure.

The anomalous initial titration curves obtained (see Figs. 1 and 2) are important confirmatory

evidence for the results obtained with the deoxypentose nucleic acid of calf thymus, and show that the hydrogen-bonded structure exists in other deoxypentose nucleic acids which have been prepared in the manner described by Gulland, Jordan, and Threlfall (*loc. cit.*). An attempt is being made to prepare samples of nucleic acid which have been separated from the protein by a method other than the chloroform-gel method of Sevag, Lackmann, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425) in order to ascertain whether the hydrogen bonded structure is in any way dependent on the method employed in splitting the nucleic acid from the protein.

Experimental.

The electrometric titrations were carried out according to the method described by Fletcher, Gulland, and Jordan (*J.*, 1944, 33), and the titration curves were corrected for the titration of water at the extremes of pH by the method of Jordan and Taylor (*loc. cit.*).

The sodium salt of the deoxypentose nucleic acid of lamb thymus was prepared by Mr. C. J. Threlfall, B.Sc., by the method of Gulland, Jordan, and Threlfall (*loc. cit.*). The sodium salt of the deoxypentose nucleic acid of herring sperm was prepared by Professor M. Stacey by the method of Mirsky and Pollister (*Proc. Nat. Acad. Sci.*, 1942, **28**, 344) of which the method of Gulland, Jordan, and Threlfall (*loc. cit.*) is a modification.

Analyses.—All samples were dried at 110° in a vacuum over phosphoric oxide. The following results were obtained. Sodium salt of the deoxypentose nucleic acid of lamb thymus (Found: C, 36·3, 36·2; H, 4·2, 4·1; N, 15·6; P, 8·85; Na, 6·9, 7·0%). Sodium salt of the deoxypentose nucleic acid of herring sperm [Found: C, 34·3; H, 3·5; N, 15·3; P, 8·8. Calc. for a long polynucleotide consisting of the tetrasodium salt of tetranucleotides containing, on the average, 1 mol. each of guanine, thymine, cytosine, and adenine deoxypentose nucleotides, *i.e.*, $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_x$: C, 35·4; H, 3·4; N, 15·9; P, 9·4%]. The additional HONa atoms of the two terminal nucleotides of a straight-chain polynucleotide have been ignored since their contribution is negligible if the polynucleotide is large.

We wish to record our thanks to the British Empire Cancer Campaign for a maintenance grant to one of us (D. J. C.), and to Professor M. Stacey for the gift of the sample of the sodium salt of the deoxypentose nucleic acid from herring sperm.

The University, Nottingham. [*Received, November 15th*, 1948.]

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302. *Reformatsky Reactions with Methyl γ-Bromocrotonate.*

By E. R. H. Jones, D. G. O'Sullivan, and M. C. Whiting.

Reformatsky reactions between methyl γ-bromocrotonate and various carbonyl compounds have been investigated. A detailed study of the reaction with benzaldehyde has revealed that distillation of the crude product at very low pressures and hydrolysis in very dilute alkaline solutions are essential in order to obtain reasonable yields of the expected δ-hydroxy-acid.

A by-product, identified as the methyl ester of β-hydroxy-β-phenyl-α-vinylpropionic acid, and methyl crotonate, which have been isolated in addition to the normal product, probably arise from different modes of reaction of the resonating anion of the initially formed zinc complex with benzaldehyde.

In view of the appearance of a paper by English and Gregory (*J. Amer. Chem. Soc.*, 1947, **69**, 2123) describing Reformatsky reactions between ethyl γ-bromocrotonate and various aldehydes, an account of some work along similar lines carried out in these laboratories seemed desirable. We have been interested in this reaction for various reasons—its application to the synthesis of vitamin A acid and related substances (Arens and van Dorp, *Rec. Trav. chim.*, 1946, **65**, 338; Heilbron, Jones, and O'Sullivan, *J.*, 1946, 866; Heilbron, *J.*, 1948, 386), the possibility of employing it for the synthesis of αβ-unsaturated δ-lactones (Haynes and Jones, *J.*, 1946, 954), and as a possible approach to the synthesis of auxin-a (Kögl and Erxleben, *Z. physiol. Chem.*, 1934, **227**, 51) and analogues.

The crude *methyl* ester of 4-phenylbut-1-en-4-ol-1-carboxylic acid (I) obtained from a Reformatsky reaction between benzaldehyde and methyl γ-bromocrotonate underwent appreciable dehydration on distillation at 0·1 mm. to cinnamylideneacetic ester (cf. English and Gregory, *loc. cit.*), the content of the latter increasing from 7% to 20%, as measured by the intensity of light absorption at 3050 A. Distillation in a short-path still at 10^{-4} mm. effectively prevented dehydration. The resulting ester was hydrolysed with a 1% solution of potassium hydroxide in aqueous methanol to the crystalline hydroxy-acid, m. p. 115°, the yield from benzaldehyde being 25%. It was shown spectrographically that in more concentrated solutions extensive dehydration occurred, cinnamylideneacetic acid being the only isolable product.

The low yields of analytically pure ester and of crystalline acid obtained by English and

Gregory (*loc. cit.*) (22%, and 7% of impure material, respectively, from benzaldehyde) are in accordance with our observations on the instability of these substances towards high temperatures and strongly basic reagents.

Ziegler *et al.* (*Annalen*, 1942, **551**, 80) stated that when benzaldehyde was condensed with methyl γ-bromocrotonate and the crude product was distilled at 12 mm. (b. p. 160—180°) it gave " 4-phenylbut-1-en-4-ol-1-carboxylic ester " in 70% yield. In our experience the yield is only of the order of 40—45% even when the crude product is distilled at 10^{-4} mm., falling to about 25% when a pressure of 0·1 mm. is used, much dehydration then taking place. Ziegler *et al.* recorded no physical constants other than boiling point, and no analysis other than a methoxyl determination not very near to the calculated value for the hydroxy-ester.

A small quantity of a by-product, m. p. 83°, was isolated on one occasion from the distilled ester; it analysed as $C_{12}H_{14}O_3$, *i.e.*, it was isomeric with the normal hydroxy-ester, it showed no high-intensity light absorption above 2200 A., and in a Zerewitinoff determination it evolved methane corresponding to the presence of one active hydrogen atom. Hydrogenation with a palladium catalyst gave an ester, m. p. 40—41°, hydrolysed by cold dilute potassium hydroxide solution to an acid, $C_{11}H_{14}O_3$, m. p. 115—116°, which proved to be β-hydroxy-β-phenyl-α-ethylpropionic acid; the literature m. p.s of 110—112° and 111·5—112·5° for the latter probably refer to mixtures of stereoisomerides. The *methyl* ester of the authentic acid had m. p. 42° and did not depress the m. p. of the hydrogenation product. Ozonolysis of the original ester, m. p. 83°, gave a 34% yield of formaldehyde; it is therefore *methyl β-hydroxy-β-phenyl-α-vinylpropionate* (II).

There are two possible explanations for the formation of this product. It might be formed by a Reformatsky reaction between benzaldehyde and methyl α-bromo-α-vinylacetate, the latter being present as an impurity in the methyl bromocrotonate used (it could be formed by an allylic rearrangement of the latter). This possibility is extremely unlikely in view of the fact that the bromocrotonic ester employed was carefully fractionated before use (it had b. p. 98·5—100°/24 mm., $n_D^{23\cdot5°}$ 1·4983—1·5003). Alternatively, the by-product might be formed from γ-bromocrotonic ester in the manner described below.

Under the same conditions crotonaldehyde gave a crude product which on distillation at 13 mm. gave the methyl ester of hepta-1 : 3 : 5-triene-1-carboxylic acid, m. p. 75—76° (Kuhn and Hoffer, *Ber.*, 1930, **63**, 2164). Fractionation of the crude ester at 1 mm., however, gave a homogeneous product in 22% yield which, according to analytical and spectrographic data, is *methyl hepta-1 : 5-dien-4-ol-1-carboxylate.*

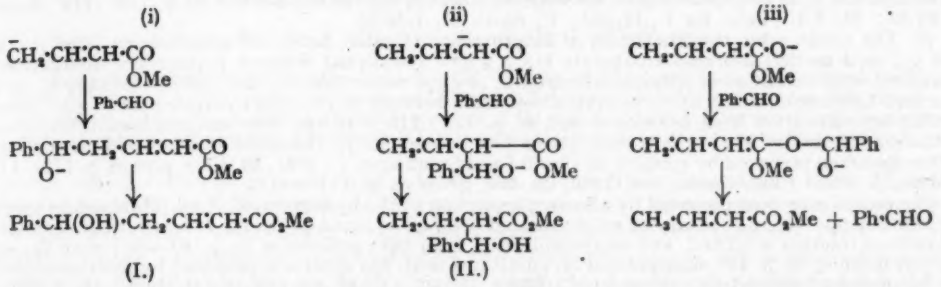
With simpler carbonyl compounds, including acetone, butaldehyde, and di*iso*propyl ketone, the results were less satisfactory. Small yields of inhomogeneous materials were obtained, and much self-condensation of the carbonyl compound appeared to take place; mesityl oxide and 2-ethylhexenal respectively were isolated, as 2 : 4-dinitrophenylhydrazones, from the low-boiling fractions in the first two cases.

The reactions with acetophenone and *cyclo*hexanone were also studied, in connection with polyene syntheses, the interest centering on the dehydrated products rather than the hydroxy-esters. In both cases the hydroxy-ester was the primary product, as shown by the absorption spectrum. The crude 4-phenylpent-1-en-4-ol-1-carboxylic ester (from acetophenone) underwent dehydration on distillation (b. p. 125—130°/10^{-3} mm.), and alkaline hydrolysis gave crystalline *δ-phenylsorbic acid* (20% yield from acetophenone).

The crude hydroxy-ester from *cyclo*hexanone was partially dehydrated with potassium hydrogen sulphate, and hydrolysis then yielded a mixture of the *hydroxy-acid* and *cyclohexylidenecrotonic acid*, separable by chromatography (yields from *cyclo*hexanone 1·2% and 2·5%, respectively). The hydroxy-ester underwent some fission on hydrolysis with hot methanolic potassium hydroxide solution into *cyclo*hexanone and crotonic acid; the former was isolated as its 2 : 4-dinitrophenylhydrazone, in 50% yield; this fact, and the difficulties of separation, account for the low yields.

In all the Reformatsky reactions methyl crotonate was formed and much of the carbonyl compound was recovered, although most of the zinc reacted. The same effect has been noted previously (Newman, *J. Amer. Chem. Soc.*, 1942, **64**, 2131) in conventional Reformatsky reactions and ascribed to enolisation of the carbonyl compound (cf. Arens and Van Dorp, *loc. cit.*; Newman, *loc. cit.*); in the case of benzaldehyde enolisation is impossible, yet methyl crotonate was formed and, despite the difficulty of separation, more benzaldehyde was recovered on one occasion than corresponded to the amount of unreacted zinc. The following alternative explanation seems plausible. The anion of the initially formed zinc complex can be supposed to react with benzaldehyde in three different ways, in accordance with the three major resonance

structures, giving, after acidification, (i) the normal product, (ii) the by-product, β-hydroxy-β-phenyl-α-vinylpropionic ester, and (iii) methyl crotonate and benzaldehyde from a readily-



hydrolysed intermediate. This hypothesis accounts convincingly for the three products isolated from the reaction between benzaldehyde and methyl γ-bromocrotonate. It gains support from the well-known behaviour of crotyl and methylvinyl carbinyl halides in Grignard reactions (*inter alia*, Young and Roberts, *J. Amer. Chem. Soc.*, 1945, **67**, 319) and the predominance of the third mode of reaction may well account for the non-isolation of any normal products from attempted Reformatsky reactions with ε-bromosorbic ester (Heilbron, Jones, and O'Sullivan, *loc. cit.*). It may also be noted that Arens and Van Dorp (*loc. cit.*) mention the isolation of isomeric by-products in Reformatsky reactions between β-ionone and methyl γ-bromocrotonate.

Several unsuccessful attempts were made to convert the 4-phenylbut-1-en-4-ol-1-carboxylic acid into the corresponding lactone. Distillation at 10^{-4} mm. and at 10 mm. did not effect any change, and treatment with acids gave cinnamylideneacetic acid. Attempts were made to isomerise this presumably *trans*-acid to the *cis*-compound by irradiation with light from a mercury-vapour lamp, and also sunlight, with and without the addition of iodine, but in all cases the original acid was recovered unchanged. Dodge's method (*J. Amer. Chem. Soc.*, 1916, **38**, 450) was also employed; a sodium hydrogen sulphite addition compound was obtained without difficulty, but attempts to convert it into the lactone failed.

The conversion of the δ-substituted δ-hydroxy-αβ-ethylenic acids into analogues of auxin-a is being studied, as also are other routes directed towards the same end. A brief account of the present work and of our future aims was given in the discussion following a paper read by the senior author at the International Congress of Pure and Applied Chemistry in London in July 1947.

Experimental.

Reformatsky Reactions.—A solution of the carbonyl compound and methyl γ-bromocrotonate (1 molecular equiv. of each) in an equal volume of dry benzene was added to the stirred suspension of activated zinc at such a rate that the benzene refluxed gently without external heating. The mixture was then heated under reflux for a further 15 minutes after the end of the spontaneous reaction. The cooled solution was worked up in the usual manner; evaporation of the benzene gave an oil containing methyl crotonate, recovered carbonyl compound, the required product, and usually an appreciable non-volatile residue.

4-Phenylbut-1-en-4-ol-1-carboxylic Acid.—The crude ester, obtained as described from benzaldehyde (21·2 g.), methyl γ-bromocrotonate (35·8 g.), and zinc (13·0 g.; 1·8 g. recovered) was heated at 100°/10^{-3} mm. to remove benzaldehyde, and distilled at 100—110° (bath temp.)/10^{-4} mm. The distillate (17·5 g.) solidified partly; after the addition of ether and light petroleum (b. p. 40—60°) the by-product (*methyl β-hydroxy-β-phenyl-α-vinylpropionate*) (0·9 g.) was separated as needles, m. p. 81°, which after crystallisation from light petroleum (b. p. 40—60°) had m. p. 83° (Found: C, 69·75; H, 6·7. $C_{13}H_{14}O_3$ requires C, 69·85; H, 6·85%). Light absorption in alcohol: Maximum, 2580 A.; $\varepsilon = 140$; inflexion, 2640 A.; $\varepsilon = 130$.

The filtrate was evaporated, the residue ($n_D^{20°}$ 1·552) dissolved in methanol (300 c.c.), and a solution of potassium hydroxide (6 g.) in water (350 c.c.) added. After standing overnight the crude acid was isolated in quantitative yield as a gum, which was dissolved in warm benzene. From the cold solution the hydroxy-acid (9·2 g.) separated as a powder, m. p. 113—114°; after crystallisation from benzene or water, it formed leaflets, m. p. 115° (English and Gregory, *loc. cit.*, give m. p. 115—116°) (Found: C, 68·75; H, 6·3. Calc. for $C_{11}H_{12}O_3$: C, 68·75; H, 6·3%). Light absorption in alcohol: Maxima 2170 and 2640 A.; $\varepsilon = 18{,}000$ and 270, respectively. The *methyl* ester, prepared by treating the sodium salt with methyl sulphate, had b. p. 149°/10^{-1} mm., $n_D^{18°}$ 1·5410 (Found: C, 69·95; H, 6·75. $C_{12}H_{14}O_3$ requires C, 69·85; H, 6·85%). Light absorption in alcohol: Maximum, 2180 A.; $\varepsilon = 17{,}000$.

β-Hydroxy-β-phenyl-α-ethylpropionic Ester and Acid.—(*a*) The by-product ester, m. p. 83° (134 mg.), was dissolved in methanol (3 c.c.) and shaken in hydrogen together with a palladium–barium sulphate catalyst (15 mg.; 5%) until absorption was complete. After filtration and evaporation of the solvent,

the residue solidified, m. p. 40—41°; a small sample (*ca.* 20 mg.) was removed and the remainder was treated with a solution of potassium hydroxide (150 mg.) in aqueous methanol (14 c.c.) for 24 hours at 20°. Acidification and isolation with ether in the usual way yielded a syrup which solidified on standing; crystallisation from *cyclo*hexene gave the hydroxy-acid (75 mg.) as fine needles, m. p. 115—116° (Found: C, 67·85; H, 7·2. Calc. for $C_{11}H_{14}O_3$: C, 68·0; H, 7·25%).

(*b*) The crude ester resulting from a Reformatsky reaction between benzaldehyde (10·6 g.), zinc (6·5 g.), and methyl α-bromo-*n*-butyrate (15 g.) was hydrolysed without preliminary distillation by treatment with a solution of potassium hydroxide (4 g.) in water (250 c.c.) and methanol (150 c.c.) at 20°. The acid fraction solidified and was crystallised from benzene to give the hydroxy-acid (6 g.). After a further crystallisation from benzene it had m. p. 110—112°; it was then recrystallised from aqueous methanol and finally from *cyclo*hexene, giving fine needles, m. p. 115—116° undepressed on admixture with a specimen prepared by method (*a*) (Perkin and Stenhouse, *J.*, 1891, **59**, 1009, give m. p. 110—112°; Andres, *J. Russ. Phys. Chem. Soc.*, 1896, **28**, 668, gives m. p. 111·5—112·5°).

The *methyl* ester was prepared by allowing a solution of the hydroxy-acid (1 g.) [obtained by method (*b*)] in methanol (10 c.c.) containing sulphuric acid (0·05 g.) to stand at 20° for 72 hours. After isolation the neutral fraction solidified, and recrystallisation from light petroleum (b. p. 40—60°) gave the ester as stout needles, m. p. 42°, undepressed on admixture with the specimen prepared by hydrogenation of the by-product [method (*a*)] (Found: C, 69·5; H, 7·9. $C_{11}H_{14}O_3$ requires C, 69·2; H, 7·75%).

Ozonolysis of Methyl β-Hydroxy-β-phenyl-α-vinylpropionate.—The ester (74·5 mg.) was dissolved in glacial acetic acid (3 c.c.) and treated with ozonised oxygen for ½ hour. Decomposition of the ozonide with zinc dust and water, followed by distillation of the aqueous solution gave 13 c.c. of liquid. Half of this was treated with a solution of "dimedone" (*ca.* 70 mg.) in cold water (15 c.c.). After standing overnight at 0°, the separated derivative (16 mg.), m. p. 160—180°, was collected. Following crystallisation from aqueous methanol it had m. p. 187°, undepressed on admixture with an authentic specimen of the formaldehyde derivative.

Methyl Hepta-1 : 5-dien-4-ol-1-carboxylate.—A Reformatsky reaction was effected between crotonaldehyde (7 g.) and methyl γ-bromocrotonate (18 g.) with zinc (6·6 g.) in benzene (25 c.c.). Fractional distillation of the product gave the *methyl* ester (3 g.), b. p. 65°/1 mm., $n_D^{21°}$ 1·4672 (Found: C, 62·9; H, 8·0. $C_9H_{14}O_3$ requires C, 63·55; H, 8·3%).

δ-Phenylsorbic Acid.—The crude ester from acetophenone (20 g.), methyl γ-bromocrotonate (30 g.) and zinc (13 g., 3·5 g. recovered) weighed 25 g. after removal of acetophenone by distillation at 1 mm. A portion (15 g.) was fractionally distilled at 10^{-2} mm.; the fraction, b. p. 125—130° (11 g.), was hydrolysed with cold 10% methanolic potassium hydroxide solution for 24 hours. Treatment of the acid fraction (5 g.) with light petroleum (b. p. 40—60°) gave the *acid* (3·5 g.) as plates, m. p. 141°, unchanged on recrystallisation (Found: C, 76·6; H, 6·45. $C_{12}H_{12}O_2$ requires C, 76·6; H, 6·45%). Light absorption in alcohol: Maxima, 2280 and 3050 A.; $\varepsilon = 10{,}500$ and 30,000 respectively. The *methyl* ester (2·3 g.), prepared in the usual way from the acid (3 g.), methanol (25 c.c.), and sulphuric acid (0·5 g.), had b. p. 130°/10^{-2} mm., $n_D^{17°}$ 1·6225 (Found: C, 76·9; H, 6·95. $C_{13}H_{14}O_2$ requires C, 77·2; H, 7·0%).

cyclo*Hexylidenecrotonic Acid* and γ-1-*Hydroxy*cyclo*hexylcrotonic Acid.*—The crude ester from *cyclo*hexanone (20 g.), methyl γ-bromocrotonate (35 g.), and zinc (13 g.; 1·5 g. recovered) was heated in nitrogen with fused potassium hydrogen sulphate (9 g.) for two hours at 160° and then for one hour at 180°. The mixture was cooled, water added, and the ester extracted with ether and washed with sodium hydrogen carbonate solution. Evaporation of the dried extract and distillation at 0·1 mm. gave the mixed esters, b. p. 90—125° (17·7 g.), which were hydrolysed with a slight excess of cold 10% methanolic potassium hydroxide for 24 hours. In this way a red solid (7·1 g.) was obtained which was sublimed at 10^{-4} mm. and crystallised from pentane to give a colourless product (5·5 g.), m. p. 58—60°. Analytical and light-absorption data indicated that this was a mixture; it was therefore chromatographed on silica gel, and two fractions were obtained: (i) m. p. 69° (1·6 g.); (ii) m. p. 58—60° (0·6 g.). On crystallisation from pentane these gave respectively cyclo*hexylidenecrotonic acid* (0·8 g.) (A) as prisms, m. p. 75° (Found: C, 71·9; H, 8·45. $C_{10}H_{14}O_2$ requires C, 72·3; H, 8·5%), and γ-1-*hydroxy*cyclo*hexylcrotonic acid* (0·4 g.) (B), as needles, m. p. 64° (Found: C, 65·0; H, 8·65. $C_{10}H_{16}O_3$ requires C, 65·2; H, 8·75%). Light absorption of (A) in alcohol: Maximum, 2680 A.; $\varepsilon = 20{,}000$. The *methyl* ester of (A), prepared in the usual way from the acid (2 g.), methanol (20 c.c.), and sulphuric acid (0·2 g.), had b. p. 100°/10^{-1} mm., $n_D^{22°}$ 1·5096 (Found: C, 73·1; H, 8·9. $C_{11}H_{16}O_2$ requires C, 73·3; H, 8·95%).

One of the authors (M. C. W.) thanks the Medical Research Council for a maintenance grant, and another (D. G. O'S.) is indebted to the University of London for a Postgraduate Studentship.

Imperial College of Science and Technology, London, S.W.7.
The University, Manchester, 13. [*Received, December 2nd,* 1948.]

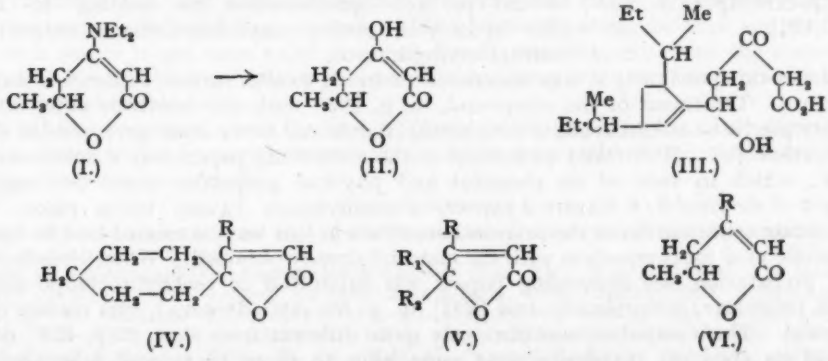
303. *Studies on Compounds Related to Auxin-a and Auxin-b. Part I.* (*Unsaturated Lactones. Part III.*)

By E. R. H. Jones and M. C. Whiting.

Acidic hydrolysis of some of the β-substituted αβ-ethylenic lactones (*e.g.*, I) described in the preceding paper, under carefully chosen conditions, leads to tetronic acids and hydroxydihydro-2-pyrones (*e.g.*, II). The light-absorption properties of these compounds are discussed and, because of its structural relationship to auxin-b (III), some of the reactions of (II) have been studied.

Moureu and Lazennac (*Compt. rend.*, 1906, **143**, 596; *Bull. Soc. chim.*, 1906, **35**, 1190) found that the adducts obtained from phenylpropiolic ester and various amines were converted on acidic hydrolysis, even under very mild conditions, into benzoylacetic ester. Similar treatment of the β-substituted αβ-ethylenic-γ- and δ-lactones (*e.g.*, I), convenient preparations of which are described in the succeeding paper, should lead to tetronic acids and 4-hydroxy-5 : 6-dihydro-2-pyrones (*cyclo*homotetronic acids) (*e.g.*, II), respectively. The former are comparatively inaccessible and no general method for the synthesis of the latter, which are related to auxin-b (III) as formulated by Kögl and his collaborators (*Z. physiol. Chem.*, 1934, **225**, 215), has yet been described. The possibility of effecting such conversions has therefore been examined.

When the lactone (IV; R = NEt_2) was treated with alcoholic oxalic acid, according to the procedure of Moureu and Lazennac (*loc. cit.*), or with cold dilute sulphuric acid, no reaction took place. (Reasons for this resistance to hydrolytic fision have been suggested in the preceding paper.) To effect hydrolysis it was found necessary to heat the lactone with an excess of concentrated hydrochloric acid at 100°, whereupon the *tetronic acid* (IV; R = OH), m. p. 198°, was obtained in 90% yield. Its structure was confirmed by its methylation with methyl



(I.) (II.) (III.)

(IV.) (V.) (VI.)

iodide and sodium hydroxide to (IV; R = OMe) (cf. succeeding paper). Similar treatment of (V; $R_1 = R_2 = Me$, R = $[CH_2]_5$>N) gave the known γγ-dimethyltetronic acid in 80% yield. In the case of the lactone (V; $R_1 = H$; $R_2 = Pr$, R = NEt_2), derived from a secondary acetylenic carbinol, it was found necessary to minimise the further hydrolysis of the primary product by employing only the calculated quantity of acid, isolating the product before the reaction was complete, and rehydrolysing the unchanged amino-lactone, in order to obtain a satisfactory yield (60%) of *hex-1-ene-2 : 3-diol-1-carboxylic acid 1→3-lactone* (*γ-propyltetronic acid*) (V; $R_1 = H$; $R_2 = Pr$; R = OH), m. p. 82°. The methoxy-lactones (IV; R = OMe; and V; $R_1 = R_2 = Me$; R = OMe) also underwent hydrolysis to the corresponding tetronic acids on drastic treatment with hydrochloric acid, but the yields were unsatisfactory. Only incomplete hydrolysis to the tetronic acid was observed when (IV; R = OMe) was heated with sodium hydroxide solution. Some of the tetronic acid was obtained from the *trans*-hydroxy-acid, $[CH_2]_5$>C(OH)·C(OPh):CH·CO_2H, on hydrolysis with hydrochloric acid.

The hydrolysis of (VI; R = NEt_2) was examined in the hope of finding a general method applicable to the synthesis of 4-hydroxy-5 : 6-dihydro-2-pyrones, which are analogues of the hypothetical lactone of auxin-b, as formulated by Kögl. It is reasonable to expect that such lactones should be convertible into δ-hydroxy-β-keto-acids analogous to auxin-b (III) itself.

Hydrolysis of the amino-lactone with concentrated hydrochloric acid at 100° gave only steam-volatile ketonic material and carbon dioxide. With 2N-hydrochloric acid [in which

(VI; R = NEt_2) is readily soluble] at 20°, an appreciable quantity of syrupy acidic material was obtained, from which a solid, m. p. 118°, could be separated. This exhibited the chemical properties expected of the desired 2-pyrone derivative (II), but the yield was only 5%. Attempts were made to improve the procedure by using continuous extraction to isolate the very water-soluble product, sulphuric or phosphoric acid then being employed. Apparently the product was sensitive to prolonged heating in ethereal solution in the presence of traces of mineral acids, since acidic syrups were obtained which, although giving strong ferric chloride colorations, failed to yield any crystalline material. Similar results followed attempts to use acetic or chloroacetic acid to effect hydrolysis, and the action of a methanolic solution of oxalic acid dihydrate, though giving diethylamine hydrogen oxalate, m. p. 209° (decomp.), in 75% yield, did not afford any of the product, m. p. 118°. It is assumed that in all these cases the 2-pyrone derivative was formed but underwent further reactions with the solvent, or self-condensation. Attention was then directed to the hydrolysis of the corresponding methoxylactone (VI; R = OMe). It was found that treatment of a solution in dry ether with carefully controlled quantities of concentrated hydrochloric acid gave a 75% yield of *4-hydroxy-6-methyl-5:6-dihydro-2-pyrone* (II), m. p. 123°, not significantly depressed on admixture with the somewhat impure material, m. p. 118°, mentioned above. The light-absorption properties of the two products were identical.

The general chemical properties and the light absorption (see below) of this substance were in agreement with the suggested structure, but with 2 : 4-dinitrophenylhydrazine, in contrast to the tetronic acids, it formed a derivative very readily. This derivative evolved carbon dioxide on recrystallisation from glacial acetic acid, giving the 2 : 4-dinitrophenylhydrazone of ethylideneacetone. This fact, and especially observations on analogous substances (Henbest and Jones, forthcoming publication), suggested that the compound, m. p. 123°, might possibly be crotonylacetic acid (VII). Although stable at 100°, it did in fact decarboxylate on heating to 200° or on acidic hydrolysis, giving ethylideneacetone, isolated as its 2 : 4-dinitrophenylhydrazone.

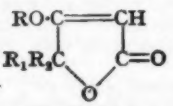
$CH_3 \cdot CH{:}CH \cdot CO \cdot CH_2 \cdot CO_2H$
(VII.)

In view of this possibility it was considered desirable to seek further evidence in support of the structure. Treatment of the compound, m. p. 123°, with diazomethane failed to yield a satisfactory product and methylation with methyl iodide and silver oxide gave, not the expected *O*-methyl ether (VI; R = OMe) (described in the succeeding paper) but a substance, m. p. 148—149°, which in view of its chemical and physical properties must be regarded as *4-hydroxy-3:6-dimethyl-5:6-dihydro-2-pyrone*, *C*-methylation having taken place. Having failed to obtain confirmation of the proposed structure in this way, it seemed best to synthesise crotonylacetic acid for comparison with the material already obtained. When β-methoxysorbic acid (for preparation, see succeeding paper) was submitted to the hydrochloric acid–ether hydrolysis procedure, *crotonylacetic acid* (VII), m. p. 68—72° (decomp.), was readily obtained in 80% yield. This compound was obviously quite different from that, m. p. 123°, described above, and its chemical properties were more akin to those of typical β-keto-acids; for instance, it was decarboxylated very readily, and on treatment with 2 : 4-dinitrophenylhydrazine hydrochloride solution yielded the derivative of ethylideneacetone directly. In view of this evidence and the entirely different light-absorption properties (see below) of the two compounds in question, eliminating the possibility of *cis–trans*-isomerism, the formulation (VI; R = OH) for the compound, m. p. 123°, must be regarded as established. Further confirmation is provided by the properties described below and by other synthetic work on analogous substances (Henbest and Jones, forthcoming publication).

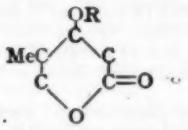
In view of its relationship to the proposed structure for auxin-b (III) (Kögl *et al.*, *loc. cit.*) the properties of the 2-pyrone derivative were examined in some detail. This dissolves with effervescence in sodium hydrogen carbonate solution and gives an immediate deep crimson colour with ferric chloride solution. Tetronic acids on treatment with sodium nitrite and then hydrochloric acid give a violet colour fading after about one minute; (VI; R = OH) gave a similar but even more transient colour. The 2 : 4-dinitrophenylhydrazone has already been mentioned and the *semicarbazone*, m. p. 200°, was obtained very readily in excellent yield. A *p-phenylphenacyl ester*, m. p. 174—174·5°, was obtained only in poor yield; it gave analyses corresponding to $C_{20}H_{18}O_4$ and presumably has the structure (VI; R = $O \cdot CH_2 \cdot CO \cdot C_6H_4 \cdot C_6H_5$). Treatment with formaldehyde and a trace of piperidine gave the *3:3'-methylenebis-(4-hydroxy-6-methyl-5:6-dihydro-α-pyrone)*, m. p. 172° (decomp.) (cf. Anschütz and Quitmann, *Annalen*, **1928**, **462**, 97).

Light-absorption data for the compounds described in this paper, together with data for

analogous substances, are presented in the accompanying table. It will be seen that the correspondence between the maxima and intensities for these hydroxy-compounds and the



Type A (5-membered ring).



Type B (6-membered ring).

Type.			$\lambda_{max.}$, A.	$\varepsilon_{max.}$
A	R = H	R_1R_2 = [CH$_2$]$_5$	2240	13,000
A	R = H	R_1 = R_2 = Me	2230	13,500
A	R = H	R_1 = Pr, R_2 = H	2230	13,500
Cf. [1] A	R = Me	R_1R_2 = [CH$_2$]$_5$	2210	15,500
B	R = H		2390	12,000
			2420	12,000
Cf. [1] B	R = Me		2340	17,000
		CH$_3$·CH:CH·C(OH):CH·CO$_2$H	2800	6,500
		Cf.[1] CH$_3$·CH:CH·C(OMe):CH·CO$_2$H	2650	15,500

[1] Succeeding paper.

related *O*-methyl derivatives described in the succeeding paper is very close, indicating that in alcoholic solutions the enolic form predominates in both cases. This supports Kummler's view (*J. Amer. Chem. Soc.*, 1938, **60**, 859; 1940, **62**, 3292) that tetronic acids exist in solution largely in the enolic form. The wave-length differences, already commented on (succeeding paper), between the 5- and 6-membered ring lactones, are again conspicuous. The data for crotonylacetic acid indicate that this substance also exists in alcoholic solution to a large extent as the enol; the $\lambda_{max.}$ difference (150 A.) between this substance and its *O*-methyl derivative is rather larger than those observed (*ca.* 20 and 60 A.) between the corresponding cyclic compounds, but the conjugation in the acyclic compounds is more extensive and hydrogen bonding is probably involved.

Work is being continued along the lines indicated in this paper and in other directions with the object of synthesising compounds with structures more closely related to those which have been given to auxins-a and -b.

Experimental.

(Except where specially referred to, the starting materials employed in the experiments below are described in the succeeding paper.)

Lactone of β-Hydroxy-β-(1-hydroxycyclohexyl)acrylic Acid (IV; R = OH).—(*a*) The lactone (IV; R = NEt$_2$) (4·6 g.) was heated on the steam-bath with concentrated hydrochloric acid (15 c.c.) for 45 minutes. The product (3·2 g.) obtained on cooling and diluting with water had m. p. 196—197°; after crystallisation from ethyl acetate the *lactone* formed prisms, m. p. 198° (2·8 g.) (Found: C, 63·95; H, 7·1. $C_9H_{12}O_3$ requires C, 64·25; H, 7·2%). Methylation: the lactone (0·5 g.), methyl iodide (2 g.), and 4N-sodium hydroxide solution (0·7 c.c.) were added to methanol (3 c.c.); after 5 days the neutral fraction was isolated with ether in the usual way; it solidified and was extracted with hot light petroleum (b. p. 40—60°); the solution on cooling yielded a solid, m. p. 90—98°, which after crystallisation from aqueous methanol had m. p. 104°, undepressed on admixture with an authentic specimen of (IV; R = OMe); the petrol-insoluble solid was crystallised from acetone–light petroleum, giving a small quantity of material, m. p. 210—215°, which was not investigated further.

(*b*) The lactone (IV; R = OMe) (1 g.) was heated under reflux with concentrated hydrochloric acid (5 c.c.) and methanol (2 c.c.) for 2½ hours. On cooling and dilution, a precipitate separated; crystallisation from ethyl acetate gave the tetronic acid (0·4 g.), m. p. 195—197°, undepressed on admixture with a specimen prepared by method (*a*).

(*c*) The lactone (IV; R = OMe) (2 g.) was heated under reflux with a solution of potassium hydroxide (1 g.) in water (10 c.c.) and methanol (4 c.c.) for 5 hours. A small amount of starting material was recovered on cooling and dilution. A solid separated from the filtrate on acidification, and after crystallisation from aqueous methanol, then ethyl acetate, the tetronic acid (*ca.* 200 mg.) was obtained as prisms, m. p. 196°, undepressed on admixture with an authentic specimen.

(*d*) *trans-β*-Phenoxy-*β*-(1-hydroxy*cyclo*hexyl)acrylic acid (100 mg.) was heated under reflux with concentrated hydrochloric acid (1 c.c.) and methanol (1 c.c.) for 2 hours. The mixture was cooled, diluted, and extracted with ether. The extract was washed thoroughly with sodium hydrogen carbonate solution and these washings gave on acidification a precipitate of the tetronic acid (*ca.* 15 mg.) which after crystallisation from ethyl acetate–light petroleum had m. p. 195—197°, undepressed on admixture with an authentic specimen.

γ-Lactone of 3-Methylbut-1-ene-2 : 3-diol-1-carboxylic Acid (*γγ-Dimethyltetronic Acid*) (V; R_1 = R_2 = Me, R = OH).—(*a*) The lactone (V; R_1 = R_2 = Me, R = N<[CH$_2$]$_5$) (1·9 g.) was heated on the steam-bath with concentrated hydrochloric acid (2 c.c.) for 45 minutes. The tetronic acid (1·0 g.),

m. p. 139—140°, separated on cooling and seeding. After crystallisation from ethyl acetate–benzene, it formed plates, m. p. 142° (Benary, *Ber.*, 1907, **40**, 1082, gives m. p. 142—143°).

(*b*) The lactone (V; $R_1 = R_2 = Me$, R = OMe) (300 mg.) was heated under reflux with concentrated hydrochloric acid (2 c.c.) for 2 hours. After cooling and addition of water (5 c.c.), the solution was extracted with ether, the extract was washed with sodium hydrogen carbonate solution, the washings were acidified, and the tetronic acid (*ca.* 50 mg.) was isolated with ether. After crystallisation from ethyl acetate–light petroleum, it had m. p. 142°, undepressed on admixture with a specimen prepared by route (*a*).

γ-Lactone of Hex-1-ene-2 : 3-diol-1-carboxylic Acid (*γ-Propyltetronic Acid*) (V; $R_1 = H$, $R_2 = Pr$, R = OH).—The lactone (V; $R_1 = H$, $R_2 = Pr$, R = NEt_2) (2·2 g.) was heated on the steam-bath with concentrated hydrochloric acid (1·1 c.c.) for 20 minutes. The solution was cooled, diluted with water, and extracted with ether. The ethereal extract was thoroughly washed with saturated sodium hydrogen carbonate solution and evaporated; the residue was heated on the steam-bath with concentrated hydrochloric acid (0·4 c.c.) for 20 minutes, cooled, diluted, and extracted with ether. This extract was well washed with sodium hydrogen carbonate solution; the combined carbonate washings were acidified and extracted with ether. The extract was dried and evaporated, and the residual oil dissolved in benzene; on adding light petroleum and seeding, a solid (1·0 g.), m. p. 80—81°, separated; after recrystallisation from water the *tetronic acid* formed plates, m. p. 83° (Found: C, 59·4; H, 7·1. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%).

4-Hydroxy-6-methyl-5 : 6-dihydro-2-pyrone (II).—(*a*) The lactone (VI; R = OMe) (810 mg.) was dissolved in dry ether (20 c.c.), concentrated hydrochloric acid (216 mg.) was added, and the mixture was set aside at 20° for 48 hours, the solid which separated being occasionally powdered by stirring with a glass rod. After cooling to 0° the *pyrone* (550 mg.) was collected; it formed small prisms, m. p. 123—124°, unchanged by recrystallisation from ethyl acetate (Found: C, 56·15; H, 6·25. $C_6H_8O_3$ requires C, 56·25; H, 6·25%). It was readily soluble in water or alcohol, less readily in ethyl acetate, and only sparingly soluble in ether or benzene. It dissolved with effervescence in sodium hydrogen carbonate solution and gave a crimson-purple colour with ferric chloride solution and an extremely transient violet colour on treatment with sodium nitrite, followed by dilute hydrochloric acid. It could be kept for some months without appreciable decomposition.

(*b*) The lactone (VI; R = NEt_2) (3 g.) was dissolved in 2N-hydrochloric acid (10 c.c.). After standing at 20° for 24 hours the solution was saturated with potassium chloride and extracted with ether (7 × 15 c.c.). The syrup obtained on evaporation of the dried extract was dissolved in benzene, and on adding light petroleum the crude hydroxy-2-pyrone (130 mg.) separated as brownish prisms, m. p. 115—116°. After crystallisation from benzene the m. p. was 118°, undepressed on admixture with an authentic specimen prepared according to route (*a*).

The 2 : 4-*dinitrophenylhydrazone*, prepared in the usual way, separated very slowly as a light brownish-yellow powder (60% yield). It decomposed without melting at 210—230°; it was almost completely insoluble in ordinary organic solvents except boiling glacial acetic acid which decomposed it rapidly (in less than 1 minute) to ethylideneacetone 2 : 4-dinitrophenylhydrazone, m. p. 158—159°, which was readily isolated by adding water. It could, however, be crystallised (with slight decomposition) from ethyl acetoacetate pre-heated to 150°; it then formed small dull-yellow laths, decomposing above 220° (Found: C, 46·85; H, 4·25. $C_{12}H_{12}O_6N_4$ requires C, 46·75; H, 3·95%).

The *semicarbazone*, prepared at room temperature in 75% yield, formed prismatic needles, m. p. 200° (decomp.), from water (Found: C, 45·35; H, 6·05. $C_7H_{11}O_3N_3$ requires C, 45·4; H, 6·0%). Light absorption: Maximum, 2650 A., $\varepsilon = 26{,}000$.

The p-*phenylphenacyl* ester, prepared in methanol in the usual way (10% yield), had m. p. 169—173°. After crystallisation from benzene–light petroleum (b. p. 80—100°) it formed fine needles, m. p. 174—174·5° (Found: C, 74·95; H, 5·4. $C_{20}H_{18}O_4$ requires C, 74·5; H, 5·65%).

3 : 3′-Methylenebis-(4-hydroxy-6-methyl-5 : 6-dihydro-α-pyrone).—The hydroxy-2-pyrone (II) (350 mg.) was dissolved in water (3 c.c.) and treated with 40% formaldehyde solution (0·15 c.c.) and a trace of piperidine. After 30 minutes at 0°, the separated product (110 mg.), m. p. 148—150°, was collected. Crystallisation from aqueous methanol and then benzene–light petroleum (b. p. 60—80°) gave the *methylene* compound of m. p. 170—172° (decomp.; variable) (Found: C, 58·25; H, 5·9. $C_{13}H_{16}O_6$ requires C, 58·2; H, 6·0%).

Ethylideneacetone.—(*a*) On hydrolysis of the 2-pyrone derivative (II) with hot 2N-sulphuric acid, ethylideneacetone was formed; it was isolated from the steam-distillate as its 2 : 4-dinitrophenylhydrazone, m. p. 158—159° (after crystallisation from ethyl acetate–methanol) undepressed on admixture with an authentic specimen.

(*b*) When heated at atmospheric pressure, (II) (100 mg.) decomposed at about 200°; a small quantity of a colourless liquid distilled, and this on treatment with 2 : 4-dinitrophenylhydrazine reagent gave the derivative of ethylideneacetone, m. p. 158—159° (after crystallisation from ethyl acetate–methanol) undepressed on admixture with an authentic specimen.

4-Hydroxy-3 : 6-dimethyl-5 : 6-dihydro-2-pyrone.—4-Hydroxy-6-methyl-5 : 6-dihydro-2-pyrone (200 mg.) was dissolved in methanol (1 c.c.); dry silver oxide (300 mg.; *ca.* 160% of theoretical) and methyl iodide (1 c.c.) were added. After 3 days at 20° the silver iodide was filtered off and the excess of methyl iodide and methanol were removed under reduced pressure, leaving a syrup which on scratching solidified partly. Trituration with benzene gave the *2-pyrone* (42 mg.; 20%), m. p. 147—148° (slight decomp.). After crystallisation from ethyl acetate it formed plates, m. p. 148—149° (slight decomp.) (Found: C, 58·9; H, 7·35. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%). It was sparingly soluble in benzene, more readily in ethyl acetate or water. It gave a purple colour with ferric chloride and dissolved with effervescence in sodium hydrogen carbonate solution. Light absorption: Maxima, 2450 and 2490 A.; $\varepsilon = 13{,}500$.

Crotonylacetic Acid.—β-Methoxysorbic acid (400 mg.) was suspended in dry ether (10 c.c.) and cooled to 0°. Concentrated hydrochloric acid (84 mg.; *i.e.*, 4 drops from a calibrated capilliary pipette) was added and the mixture was shaken occasionally. After 2½ hours at 0° the acid had dissolved completely;

the ether was then decanted from the aqueous layer and evaporated under reduced pressure. During the latter operation the product crystallised; trituration with light petroleum gave a product (280 mg.; 80%), m. p. 61—66° (decomp.). After crystallisation from light petroleum (b. p. 60—80°) containing a little benzene the *keto-acid* was obtained as prismatic needles m. p. 68—72° (decomp.) (Found: C, 56·05; H, 5·95. $C_6H_8O_3$ requires C, 56·25; H, 6·25%). It was readily soluble in the usual organic solvents other than light petroleum, gave a deep red colour with ferric chloride, and dissolved with effervescence in sodium hydrogen carbonate solution. It lost carbon dioxide very readily on heating, but could be kept at room temperature for some weeks without decomposition.

On treatment with 2 : 4-dinitrophenylhydrazine reagent (aqueous) the derivative of ethylideneacetone was obtained, m. p. 158—159° (after crystallisation from ethyl acetate–methanol) undepressed on admixture with an authentic specimen.

The authors wish to thank Professor Sir Ian Heilbron, D.S.O., F.R.S., for his interest and encouragement and Dr. E. A. Braude for light-absorption data. One of them (M. C. W.) is indebted to the Medical Research Council for a maintenance grant.

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304. *Unsaturated Lactones. Part II. (Researches on Acetylenic Compounds. Part XIX.) Reactions of the Esters of αβ-Acetylenic Hydroxy-acids with Nucleophilic Reagents.*

By E. R. H. JONES and M. C. WHITING.

With esters of both γ- and δ-hydroxy-αβ-acetylenic acids (*e.g.*, I and IV) amines give β-substituted γ- and δ-lactones (*e.g.*, II, III, and V). It is suggested that the initial reaction in these cases is *cis*-addition of the nucleophilic reagent to the triple bond. The addition of alcohols, catalysed by alkoxides or boron trifluoride–mercuric oxide, similarly leads to β-alkoxylactones (*e.g.*, II, III, and VII). With phenol and benzylthiol mixtures of lactones and (presumably) *trans*-hydroxy-esters are obtained.

The β-substituted γ-lactones exhibit anomalous light absorption properties and are unusually resistant to acid or alkaline hydrolysis. These abnormalities are explained in terms of enhanced stabilisation of dipolar resonance forms by an inductive effect through the ring system.

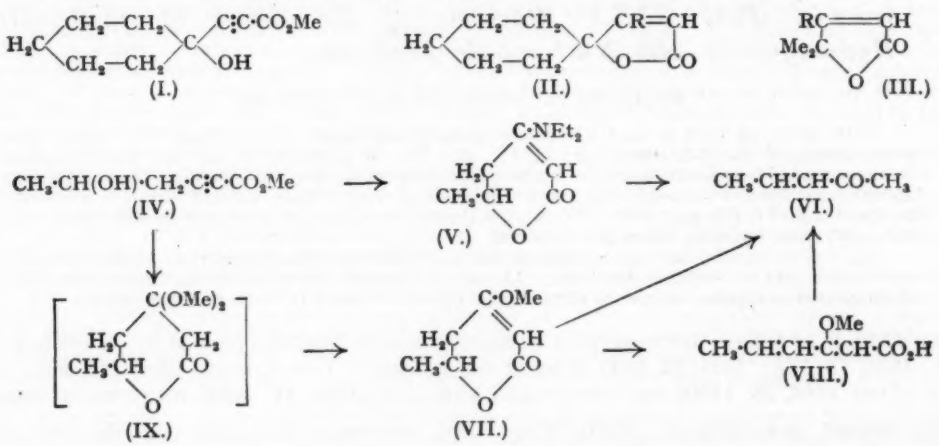
THE addition of amines to the esters of αβ-acetylenic acids has been studied by Ruhemann *et al.* (*J.*, 1898, **73**, 723; 1894, **75**, 954), Moureu and Lazennac (*Compt. rend.*, 1906, **143**, 596; *Bull. Soc. chim.*, 1906, **35**, 1190), and Strauss and Voss (*Ber.*, 1926, **59**, 1740), all of whom obtained β-substituted mono-adducts ($NHR{\cdot}\overset{|}{C}{:}CH{\cdot}CO_2Et$), sometimes formulated as the imino-compounds ($NR{:}\overset{|}{C}{\cdot}CH_2{\cdot}CO_2Et$). The application of this and analogous reactions to the esters of the readily available αβ-acetylenic γ- and δ-hydroxy-acids (Haynes and Jones, *J.*, 1946, 503, 954) has now been examined. This research was initiated with the object of obtaining amino-substituted αβ-unsaturated lactones of possible biological interest and as part of a more extensive project on the synthesis of substances related to auxins-a and -b.

Exothermic reactions occurred when the αβ-acetylenic hydroxy-esters were treated with secondary aliphatic amines at room temperature; aniline was less reactive and in this case heating to 100° was necessary to initiate reaction. Analytical data indicated that the products in all cases were lactones, and their β-substituted nature was evident from their behaviour on hydrolysis (see below and preceding paper). Thus, the *ester* (I) gave with diethylamine, diethanolamine, and aniline crystalline *lactones* [II; R = NEt_2, $N(CH_2{\cdot}CH_2{\cdot}OH)_2$, and NHPh respectively) in yields ranging from 45 to 85%. The *methyl* ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid similarly gave crystalline *lactones* (III; R = $C_5H_{10}N$ and NHPh) with piperidine and aniline (65% yield in each case), and the methyl ester of the homologous hex-1-yn-3-ol-1-carboxylic acid, derived from a secondary carbinol, gave with diethylamine a liquid *lactone* in 75% yield. The *methyl* ester of the δ-hydroxy-acid, pent-1-yn-4-ol-1-carboxylic acid (IV), and diethylamine gave with equal facility the δ-lactone (V), which is formally related to the naturally occurring δ-$\Delta^{\alpha\beta}$-hexenolactone (cf. Haynes and Jones, *loc. cit.*). Hydrolysis of the amino-lactone (V) with 20% sulphuric acid was accompanied by decarboxylation giving ethylideneacetone (VI).

In spite of a careful study in several cases, no products other than the amino-lactones could be isolated from these addition reactions. This fact appears to support the hypothesis that the initial reaction of primary and secondary amines is *cis*-addition to the acetylenic linkage, followed by lactone formation by the elimination of alcohol from the activated molecule. It is possible, however, that equilibrium between the products of *trans*- and *cis*-addition might

be facilitated by the diminution in double-bond character of the ethylenic linkage in $R_2N{-}C{=}C{-}C{=}O$, due to resonance with a form of the type $R_2\overset{+}{N}{=}C{-}C{=}C{-}\overset{-}{O}$, the stabilisation of the *cis*-adduct by lactonisation resulting in the latter being the sole product. There is some evidence of facile interconversion between the two isomers of β-anilinoacrylic ester (Strauss and Voss, *loc. cit.*) and in the case of some of the amine adducts obtained from ethynyl ketones (Bowden and Jones, *J.*, 1946, 45). It seems probable that further studies now in progress will make some contribution towards the elucidation of the mechanism of the addition of nucleophilic reagents to acetylenic linkages.

The addition of alcohols to the esters of αβ-acetylenic carboxylic acids in the presence of alkali alkoxides has been described by Michael and Bucher (*Ber.*, 1895, **28**, 2512; 1896, **29**, 1792) and Moureu (*Bull. Soc. chim.*, 1904, **31**, 293), both mono- and di-adducts being obtained, the latter, however, only under comparatively vigorous conditions. The addition of alcohols to acetylenic bonds, not necessarily conjugated to carbonyl or similar groups, in the presence of a mercuric oxide–boron trifluoride catalyst has been extensively investigated. It was applied to the esters of αβ-acetylenic acids by Hennion and Zoss (*J. Amer. Chem. Soc.*, 1941, **63**, 1151). Recently, it was employed by Raphael (*J.*, 1947, 805) in the case of the ester of a γ-hydroxy-αβ-acetylenic acid, a β-methoxy-αβ-ethylenic γ-lactone being obtained in good yield.



The ester (I) and the methyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid reacted vigorously with methanol containing sodium methoxide (30—50 mols. %) to give 60% yields of crystalline *lactones* (II and III, respectively; R = OMe). With the methyl ester of hex-1-yn-3-ol-1-carboxylic acid, derived from a secondary carbinol, the addition of methanol was less smooth; to avoid a very vigorous reaction leading to the formation of gummy acidic material it was essential to conduct the reaction below room temperature; even so, the yield of the liquid *methoxy-lactone* was only 20%. With the mercuric oxide–boron trifluoride catalyst, however, a 65% yield of the same product was obtained.

When (IV), a δ-hydroxy-ester, was treated with methanolic sodium methoxide at 0° it gave a mixture of a neutral product, m. p. 69°, with lactonic properties, and an acid, m. p. 152° (decomp.). Both were isomeric with the starting material and both were hydrolysed rapidly by hot 2N-sulphuric acid to ethylideneacetone (VI) (isolated as its 2 : 4-*dinitrophenylhydrazone*). There was therefore little doubt that these substances were respectively β-*methoxy*-δ-$\Delta^{\alpha\beta}$-*hexeno-lactone* (VII) and β-*methoxysorbic* acid (VIII), respectively, and this conclusion was confirmed by their light-absorption properties (see below). The variations in the yields of the two products with time of reaction and methoxide concentration are tabulated in the Experimental portion. The yield of acid increased at the expense of that of lactone with increasing methoxide concentration and, as was expected, it was found that (VIII) was formed by the action of sodium methoxide on (VII) (cf. Hofmann, *Annalen*, 1859, **110**, 129). It may be noted that δ-hydroxy-αβ-ethylenic esters are similarly unstable, readily undergoing dehydration under alkaline conditions (Jones, O'Sullivan, and Whiting, this vol., p. 1415).

This method thus seemed to be unsatisfactory for preparing the lactone (VII), and the effect of employing the boron trifluoride–mercuric oxide catalyst was therefore examined. On mixing the reactants and the catalyst a vigorous exothermic reaction took place; isolation of the

product shortly after this had subsided gave a non-homogeneous oil, b. p. *ca.* 80°/0·1 mm., which contained *ca.* 35% of (VII), estimated by light-absorption data, and no other light-absorbing substance and none of the starting material (shown by the absence of an exothermic reaction with piperidine). On standing for 24 hours at room temperature with a methanolic solution of boron trifluoride or of sulphuric acid, it was converted into crystalline (VII), which was also obtained, in 65% yield, when the original reaction mixture was allowed to stand for 24 hours or more before isolation of the product. The first-formed oil gave, on treatment with methanolic 2 : 4-dinitrophenylhydrazine sulphate solution, the derivative of 4-hydroxy-6-methyl-5 : 6-dihydro-2-pyrone (see the preceding paper) in good yield; this was not obtained under the same conditions from (VII). It is accordingly considered that the liquid product consists largely of the dimethoxy-lactone (IX), a view which gains support from the fact that the simple acetylenic esters give di-adducts with the boron trifluoride–mercuric oxide catalyst, which lose one molecule of methanol on vigorous acid treatment (Hennion and Zoss, *loc. cit.*). The β-substituted δ-lactones of type (VII) are thus, as in many other respects (see below), intermediate in behaviour between the γ-lactones and the analogous open-chain compounds. By the same procedure the methyl ester of but-1-yn-4-ol-1-carboxylic acid gave the corresponding *lactone*, m. p. 78°, in similar yield.

The ester (I) on treatment with ethylene glycol in which a small quantity of sodium had been dissolved gave the *lactone* (II; R = O·CH_2·CH_2·OH) in 45% yield. The ethyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid gave a 20% yield of the corresponding *ethoxy-lactone* with ethanolic sodium ethoxide. The addition of primary alcohols to the esters of αβ-acetylenic hydroxy-acids, under the influence of alkoxide or boron trifluoride–mercuric oxide catalysts, and leading to β-substituted αβ-ethylenic γ- and δ-lactones, thus appears to be a general reaction. Attempts to utilise secondary alcohols, *e.g.*, *iso*propyl alcohol and *cyclo*hexanol, with alkoxide catalysis were uniformly unsuccessful, this paralleling the experience of Bowden and Jones (unpublished results) in attempts to add similar alcohols to ethynyl ketones.

The addition of phenols to the esters of αβ-acetylenic acids in the presence of the appropriate alkali phenoxides was examined by Ruhemann and Beddow (*J.*, 1900, **77**, 984), employing the esters of phenylpropiolic and acetylenedicarboxylic acids. They obtained β-substituted mono-adducts, probably formed by *trans*-addition.

When the ester (I) was treated with phenol and sodium phenoxide at *ca.* 80°, an exothermic reaction took place and two crystalline compounds, m. p.s 103° and 69·5°, readily separated by fractional crystallisation, were isolated from the neutral portion of the product (total yield, 70%). The former product proved to be the *hydroxy*-ester $[CH_2]_5 > C(OH)\cdot C(OPh){:}CH\cdot CO_2Me$, hydrolysed by cold alkali to the *hydroxy-acid*, which showed no tendency to cyclise to a lactone even when heated above its melting point. It seems reasonable to assume that in the ester the carbomethoxyl and the 1-hydroxy*cyclo*hexyl group have a *trans*-configuration about the double bond. The second compound, m. p. 69·5°, is the *lactone* (II; R = OPh). No evidence of the formation of the latter from the hydroxy-ester could be obtained by treatment with potassium phenoxide and phenol even under conditions much more vigorous than those appertaining in the addition reaction, and it is concluded that the hydroxy-ester and the lactone are formed independently by simultaneous *trans*- or *cis*-additions, or by exclusive *cis*-addition followed by either isomerisation or cyclisation of an unstable *cis*-adduct.

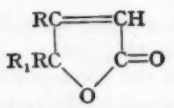
The addition of thiophenols to αβ-acetylenic esters was studied by Ruhemann *et al.* (*J.*, 1900, **77**, 1179), who used the corresponding sodium salt as catalyst, and obtained mainly β-substituted mono-adducts. Secondary and tertiary amines have been used to catalyse analogous addition reactions to αβ-acetylenic and -ethylenic carbonyl compounds. The ester (I) reacted exothermically with benzylthiol in the presence of a little triethylamine. When the product, an uncrystallisable syrup, was hydrolysed with cold potassium hydroxide solution, the *lactone* (II; R = Ph·CH_2·S) and the corresponding *hydroxy-acid* (presumably *trans*) were readily isolated from the neutral and the acidic fraction, respectively, in a total yield of about 80%.

When the light absorption properties of the lactones and hydroxy-acids described in this paper are compared with those of corresponding acyclic analogues some interesting anomalies are observed (see Table). Whereas the data for δ-lactones of type B show little variation from those of the simple β-substituted crotonic acid derivatives the γ-lactones (type A) absorb at much shorter wave-lengths. The effect is particularly marked in the β-dialkylamino-series, where the differences average about 200 A. These light-absorption anomalies are almost certainly related to the remarkable chemical stability which these γ-lactones exhibit. Acid hydrolysis to tetronic acids (preceding paper) takes place only under drastic conditions, whereas acyclic analogues, *e.g.*, β-diethylamino-αβ-ethylenic esters, undergo rapid hydrolysis even with organic

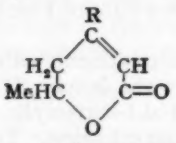
acids (Moureu and Lazennac, *loc. cit.*). The non-formation of di-adducts in alkoxide-catalysed alcohol addition reactions, the almost complete absence of basic properties in the amino-lactones and the impossibility of preparing methiodides from the latter, are further examples. Abnormal stability is also observed on attempted hydrolytic fission of the 5-membered lactone ring; after standing in 10% potassium hydroxide in methanol for some days the lactone (II; R = OMe) was recovered in 75% yield on dilution and ether extraction. The δ-lactones, which do not exhibit any light-absorption anomalies, are much more normal in their chemical properties.

In β-substituted αβ-unsaturated esters and carbonyl compounds in which the substituent carries a free electron-pair, resonance with dipolar forms results in characteristic variochromic effects (Bowden, Braude, Jones, and Weedon, *J.*, 1946, 45). The parallelism between the auxochromic effects of such substituents and their tautomeric effects on chemical reactivity is well known (see, *inter alia*, Bowden, Braude, and Jones, *J.*, 1946, 984). The chemical and optical

Light absorption data for β-substituted αβ-unsaturated lactones and the corresponding hydroxy-acids (in alcoholic solutions).



Type A (5-membered ring).



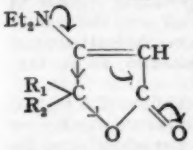
Type B (6-membered ring).

Type.			$\lambda_{max.}$, A.	$\varepsilon_{max.}$
A	R = NEt_2	$R_1R_2 = (CH_2)_5$	2680	29,000
A	R = $N(CH_2{\cdot}CH_2{\cdot}OH)_2$	$R_1R_2 = (CH_2)_5$	2680	29,000
A	R = N<[CH_2]$_5$	$R_1 = R_2 = Me$	2670	33,000
A	R = NEt_2	$R_1 = R_2 = Me$	2670	30,000
A	R = NEt_2	$R_1 = Pr$; $R_2 = H$	2650	28,000
B	R = NEt_2		2910	28,000
B	R = N<[CH_2]$_5$		2910	23,500
	cf. $Et_2N{\cdot}CMe{:}CH{\cdot}CO_2Et$ [1]		2850 2880	30,500 30,500
A	R = NHPh	$R_1R_2 = (CH_2)_5$	2810 2880	27,000 24,000
A	R = NHPh	$R_1 = R_2 = Me$	2810	24,000
	cf. $NHPh{\cdot}CMe{:}CH{\cdot}CO_2Et$ [2]		2940	11,500
A	R = OMe	$R_1R_2 = (CH_2)_5$	2210	15,500
A	R = $O{\cdot}CH_2{\cdot}CH_2OH$	$R_1R_2 = (CH_2)_5$	2210	16,000
A	R = OMe	$R_1 = R_2 = Me$	2180	17,000
A	R = OEt	$R_1 = R_2 = Me$	2190	19,000
A	R = OMe	$R_1 = Pr$; $R_2 = H$	2210	13,500
B	R = OMe		2340	17,000
B	R = OMe	without Me substituent	2330	13,500
	cf. $MeO{\cdot}CMe{:}CH{\cdot}CO_2H$ [3]		2340	13,500
A	R = OPh	$R_1R_2 = (CH_2)_5$	2230 2370 *	13,000 11,000
	[CH_2]$_5$>$C(OH){\cdot}C(OPh){:}CH{\cdot}CO_2Me$		2180 2560	21,500 3,500
	[CH_2]$_5$>$C(OH){\cdot}C(OPh){:}CH{\cdot}CO_2H$		2200 2540	16,000 3,500
	cf. $PhO{\cdot}CMe{:}CH{\cdot}CO_2H$		2360	11,000
A	R = $S{\cdot}CH_2Ph$	$R_1R_2 = (CH_2)_5$	2650	16,000
	[CH_2]$_5$>$C(OH){\cdot}C(S{\cdot}CH_2Ph){:}CH{\cdot}CO_2H$		2160 † 3000	16,000 3,000
	cf. $Ph{\cdot}CH_2{\cdot}S{\cdot}CH{:}CH{\cdot}CO_2H$ [4]	*trans*	2810	16,000
	,,	*cis*	2730	20,000
	cf. β-Methoxysorbic acid		2650	15,000
	Sorbic acid		2540	25,000

* Inflexion. † No maximum.

[1] Bowden, Braude, Jones, and Weedon, *J.*, 1946, 45.
[2] Dr. E. A. Braude, private communication.
[3] Owen, *J.*, 1945, 385.
[4] Dr. L. N. Owen and M. Sultanbawa, private communication.

peculiarities of the β-substituted 5-membered-ring lactones, as compared with those of their acyclic analogues, can be ascribed to increased dipolar character arising from the proximity of the electropositive substituent R and the electronegative carboxyl residue. This results in the electron drift due to the tautomeric effect being supplemented by an inductive effect operating along the alternative path around the ring (see inset). Such increased dipolar character would clearly lead to a diminished tendency towards proton addition at the nitrogen or similar atom—hence the resistance both to acid hydrolysis and to quaternary-salt formation and to reduced reactivity of the potential carboxyl group towards hydroxyl ions. As would be expected the effects are less marked in systems with β-substituents (*e.g.*, NHPh, OMe) containing less mobile electron pairs, and they largely disappear when the possibility of transmission of inductive effects is greatly reduced, in the 6-membered rings, by the introduction of a further methylene group.



The differences between the absorption spectra (Table) of the *trans*-hydroxy-acids and their esters, and those of their simple analogues are presumably due to steric inhibition of resonance, evidence in support of which has been accumulating rapidly in recent years. The effects observed (greatly reduced intensities of absorption and displacements towards longer wavelengths) are similar to those found in cases (*e.g.*, β-ionone) where substitution tends to prevent the adoption of coplanar structures in which maximum resonance is possible (see, *inter alia*, Braude, Jones, Koch, Richardson, and Sondheimer, this vol., p. 607). In the present instances the possibility of interference with the coplanarity of the $Ph\cdot CH_2\cdot S\cdot C{=}C\cdot C{=}O$ and $PhO\cdot C{=}C\cdot C{=}O$ chromophores by the bulky hydroxy*cyclo*hexyl group is readily apparent.

The availability of data for both stereoisomers of β-benzylthioacrylic acid demonstrates that the anomalies discussed are not due merely to *cis–trans*-isomerism.

Experimental.

Acetylenic Hydroxy-acids.—These were prepared essentially by the method of Haynes and Jones (*loc. cit.*) except that the solution of the Grignard complex, obtained by adding the acetylenic carbinol to the cooled solution of ethylmagnesium bromide, was not heated under reflux. The formation of the complex appears to be completed in the cold when the reaction is effected on a moderately large scale, notwithstanding the evidence of Zerewitinoff determinations (Jones and McCombie, *J.*, 1942, 733) which were carried out in di*iso*amyl ether. In this way better yields of purer products were obtained and the procedure was expedited. It was also found possible to prepare the acid derived from 1-ethynyl*cyclo*hexanol by pouring the solution of the Grignard complex in a mixture of ether and benzene (1 : 1) on to a large excess of carbon dioxide with manual stirring. The use of high-pressure equipment was thereby avoided, the conversion falling only from 70% to 50%. Ether, benzene, and toluene separately were less satisfactory solvents in this case. Propylethynylcarbinol was similarly carboxylated (30% conversion) using benzene as solvent and without stirring.

Esterifications.—The appropriate acids, the preparation of which has been described by Haynes and Jones (*loc. cit.*), were esterified in the usual manner with methanol and sulphuric acid. The *methyl* ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (I) formed needles, m. p. 59—60°, after crystallisation from benzene–light petroleum (b. p. 40—60°) (Found: C, 66·1; H, 7·85. $C_9H_{14}O_3$ requires C, 65·9; H, 7·75%). The *methyl* ester of pent-1-yn-4-ol-1-carboxylic acid (IV) had b. p. 86°/0·1 mm., $n_D^{19°}$ 1·4678 (Found: C, 59·45; H, 7·1. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%). The *methyl* ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid had b. p. 70°/0·2 mm., $n_D^{22°}$ 1·4555 (Found: C, 59·35; H, 6·95. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%). The methyl ester of pent-1-yn-4-ol-1-carboxylic acid was also prepared, with the same physical constants, by treating the product of the high-pressure carboxylation with a solution of excess of sulphuric acid in methanol (cooling), filtering from magnesium sulphate, refluxing, isolating with ether, and separating the methyl ester from the pent-1-yn-4-ol by distillation.

*Lactone of β-Diethylamino-β-(1-hydroxy*cyclo*hexyl)acrylic Acid* (II; R=NEt_2).—Diethylamine (2 g.) was added to a solution of the methyl ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (3 g.) in ether (15 c.c.). After 24 hours' standing at 20°, more diethylamine (2 g.) was added, the ether was slowly distilled off, and the residue was heated for 30 minutes on the steam-bath. On cooling, the residual oil solidified, and trituration with light petroleum (b. p. 60—80°) gave a product (3·4 g.), m. p. 108—110°. After crystallisation from benzene–light petroleum, the *lactone* formed leaflets (3·1 g.), m. p. 112° (Found: C, 70·0; H, 9·5. $C_{13}H_{21}O_2N$ requires C, 69·9; H, 9·5%).

*Lactone of β-Bis-2-hydroxyethylamino-β-(1-hydroxy*cyclo*hexyl)acrylic Acid* [II; R = $N(C_2H_4\cdot OH)_2$].—Diethanolamine (1·3 g.) was added to a solution of the methyl ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (2·1 g.) in acetone (4 c.c.). After standing for 18 hours at 20°, the separated crystals were collected and recrystallised from ethyl acetate to give the *lactone* (1·7 g.) as needles, m. p. 129° (Found: C, 61·05; H, 8·2. $C_{13}H_{21}O_4N$ requires C, 61·15; H, 8·3%).

*Lactone of β-Anilino-β-(1-hydroxy*cyclo*hexyl)acrylic Acid* (II; R = NHPh).—Aniline (0·8 g.) was added to the methyl ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (1·0 g.) and the mixture was heated for 24 hours on the steam-bath, a mass of crystals being obtained. Digestion with boiling methyl cellosolve gave the *lactone* (1·0 g.) as plates, m. p. 306°, unchanged on crystallisation from pyridine or ethyl acetoacetate (Found: C, 74·1; H, 6·95; N, 6·15. $C_{15}H_{17}O_2N$ requires C, 74·0; H, 7·05; N, 5·75%).

Lactone of 2-Piperidino-3-methylbut-1-en-3-ol-1-carboxylic Acid (III; R = NC_5H_{10}).—Piperidine (3 g.) was added to a solution of the methyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid (3 g.) in ether (15 c.c.). A vigorous exothermic reaction took place, some of the ether evaporating. After 24 hours' standing at 20°, more piperidine (2 g.) was added, the ether was slowly distilled off, and the residue was heated for 1 hour on the steam-bath. The resultant oil solidified on cooling; crystallisation from benzene–light petroleum (b. p. 60—80°) or water gave the *lactone* (3·1 g.) as long needles, m. p. 111° (Found: C, 67·9; H, 8·55. $C_{11}H_{17}O_2N$ requires C, 67·65; H, 8·75%).

Lactone of 2-Anilino-3-methylbut-1-en-3-ol-1-carboxylic Acid (III; R = NHPh).—Aniline (2·5 g.) was added to the methyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid (2·5 g.), and the mixture was heated on the steam-bath for 24 hours, a crystalline mass being obtained. Methanol (5 c.c.) was added, and the insoluble *lactone* (2·75 g.) was filtered off; m. p. 256° with slight decomposition, unchanged by recrystallisation from ethanol (Found: C, 71·2; H, 6·3. $C_{12}H_{13}O_2N$ requires C, 70·9; H, 6·45%).

Lactone of 2-Diethylaminohex-1-en-3-ol-1-carboxylic Acid.—Diethylamine (4 g.) was added to a solution of the methyl ester of hex-1-yn-3-ol-1-carboxylic acid (4 g.) (Haynes and Jones, *loc. cit.*) in dry ether (8 c.c.). An exothermic reaction took place; after 24 hours' at 20°, more diethylamine (2 g.) was added and the mixture was heated on the steam-bath for 30 minutes. After the removal of the more volatile material, distillation at 100° (bath temp.)/10^{-4} mm. gave the *lactone* (4·4 g.) as a yellow, viscous liquid, $n_D^{18°}$ 1·5168 (Found: C, 66·8; H, 9·45. $C_{11}H_{19}O_2N$ requires C, 66·95; H, 9·7%).

Lactone of 2-Diethylaminopent-1-en-4-ol-1-carboxylic Acid (V).—Diethylamine (7 g.) was added to a solution of the methyl ester of pent-1-yn-4-ol-1-carboxylic acid (6·6 g.) in dry ether (10 c.c.). An exothermic reaction took place; after standing at 20° for 24 hours the mixture was heated on the steam-bath for 30 minutes. Removal of the more volatile material and distillation at 80° (bath temp.)/10^{-4} mm. gave the *lactone* (8·8 g.), $n_D^{18°}$ 1·5230 (Found: C, 65·3; H, 9·7. $C_{10}H_{17}O_2N$ requires C, 65·55; H, 9·35%).

Hydrolysis. The lactone (30 mg.) was heated under reflux for 15 minutes with 20% sulphuric acid; water (15 c.c.) was added, and the solution was distilled. The distillate (10 c.c.) was treated with an aqueous solution of 2 : 4-dinitrophenylhydrazine hydrochloride; a precipitate was formed which became crystalline on addition of methanol and heating. Crystallisation from ethyl acetate–methanol gave the *ethylideneacetone* 2 : 4-*dinitrophenylhydrazone* as spherical aggregates of small needles, m. p. 158—159°, undepressed on admixture with an authentic specimen. The latter was prepared in the usual way from ethylideneacetone and alcoholic 2 : 4-dinitrophenylhydrazine sulphate solution; after crystallisation from ethyl acetate it had m. p. 158—159° (Found: C, 50·1; H, 4·4. $C_{11}H_{12}O_4N_4$ requires C, 50·0; H, 4·6%). Light absorption: Maxima, 2450, 2510, 2580, 2900, and 3800 A.; ε = 17,000, 17,000, 17,000, 9,500, and 26,500, respectively; inflexion, 2810 A.; ε = 12,000.

Lactone of β-Methoxy-β-(1-hydroxycyclohexyl)acrylic Acid (II; R = OMe).—The methyl ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (2 g.) was added to a solution of sodium methoxide (from 0·1 g. of sodium) in dry methanol (3 c.c.), and the solution was heated under reflux for 45 minutes. On cooling and adding water (7 c.c.) the product crystallised; recrystallisation from aqueous methanol gave the *lactone* (1·0 g.) as laths, m. p. 108° (Found: C, 65·85; H, 7·6. $C_{10}H_{14}O_3$ requires C, 65·9; H, 7·75%).

Lactone of β-2-Hydroxyethyl-β-(1-hydroxycyclohexyl)acrylic Acid (II; R = O·CH_2·CH_2·OH).—Sodium (0·1 g.) was dissolved in warm ethylene glycol (3 c.c.); to the cooled solution the methyl ester of β-(1-hydroxy*cyclo*hexyl)propiolic acid (2 g.) was added, and the mixture was heated on the steam-bath for 1½ hours. On cooling and dilution with water (5 c.c.), the product separated; recrystallisation from ethyl acetate–benzene gave the *lactone* (1·13 g.) as needles, m. p. 126° (Found: C, 62·6; H, 7·5. $C_{11}H_{14}O_4$ requires C, 62·25; H, 7·6%).

Lactone of 2-Methoxy-3-methylbut-1-en-3-ol-1-carboxylic Acid (III; R = OMe).—The methyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid (3 g.) was added to a solution of sodium methoxide (from 0·3 g. of sodium) in dry methanol (6 c.c.). After the initial vigorous reaction had subsided the mixture was heated under reflux for 45 minutes, diluted with water (30 c.c.), and extracted thoroughly with ether. Evaporation of the dried extract gave an oil which solidified on cooling and was crystallised from a small quantity of water to give the *lactone* (1·8 g.), m. p. 73—74°, which after recrystallisation from benzene–light petroleum formed prisms, m. p. 74° (Found: C, 59·35; H, 7·2. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%).

The corresponding *ethoxy-lactone* was obtained in the same way from the ethyl ester of 3-methylbut-1-yn-3-ol-1-carboxylic acid (Haynes and Jones, *loc. cit.*) (6 g.), sodium ethoxide, and ethanol, as an oil, b. p. 72°/0·01 mm., which after distillation solidified and was crystallised from light petroleum, giving prisms (1·3 g.), m. p. 40° (Found: C, 61·65; H, 7·55. $C_8H_{12}O_3$ requires C, 61·55; H, 7·75%).

Lactone of 2-Methoxyhex-1-en-3-ol-1-carboxylic Acid.—(*a*) The methyl ester of hex-1-yn-3-ol-1-carboxylic acid (6 g.) (Haynes and Jones, *loc. cit.*) was added to a cooled solution of sodium methoxide (from 0·7 g. of sodium) in dry methanol (30 c.c.). The mixture was kept at 0° for 6 hours and then at 20° for 18 hours. A solution of potassium hydroxide (3 g.) in water (7 c.c.) was then added, and the mixture kept for a further 24 hours at 20° to hydrolyse any unchanged ester. After addition of water (100 c.c.), the solution was extracted with ether; the dried extract was evaporated and the residual oil was distilled, giving the *lactone* (1·3 g.), b. p. 83°/10^{-3} mm., $n_D^{20°}$ 1·4724 (Found: C, 61·9; H, 7·8. $C_8H_{12}O_3$ requires C, 61·5; H, 7·75%).

(*b*) Boron trifluoride–ether complex (0·3 g.), trichloroacetic acid (0·1 g.), and mercuric oxide (0·3 g.) were added to dry methanol (6 c.c.). The mixture was heated to 65°, then cooled to 30°, and the methyl ester of hex-1-yn-3-ol-1-carboxylic acid (4·5 g.) was added. After standing at 20° for 24 hours the solution was filtered without using pressure, poured into water, and extracted with ether. The dried extract was evaporated, and the residue distilled, giving the lactone (2·9 g.), b. p. 87°/0·1 mm., $n_D^{18°}$ 1·4717.

Lactone of 2-Methoxypent-1-en-4-ol-1-carboxylic Acid (VII) *and β-Methoxysorbic Acid* (VIII).—(*a*) The methyl ester of pent-1-yn-4-ol-1-carboxylic acid (3 g.) was added to a cooled solution of sodium methoxide (from 0·3 g. of sodium) in dry methanol (10 c.c.). The solution was kept for 6 hours at 0°, then for 18 hours at 20°; water was added and the solution was saturated with sodium chloride and thoroughly extracted with ether. Evaporation of the dried extract gave an oil which solidified on

cooling and was crystallised from light petroleum to give the *lactone* (510 mg.) which after recrystallisation from *cyclo*hexene formed prisms, m. p. 69° (Found: C, 59·2; H, 7·0. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%).

Acidification of the aqueous layer, after the ether extraction, gave a crystalline precipitate of *β-methoxysorbic acid* (230 mg.), m. p. 150° (decomp.). The acid decomposed on warming with water, but was recrystallised either by dissolving it in cold methanol and adding water or in the usual way from benzene; it then formed prisms, m. p. *ca.* 152° (decomp.) (Found: C, 58·8; H, 6·95. $C_7H_{10}O_3$ requires C, 59·15; H, 7·1%).

When the proportion of sodium methoxide used was increased, the yield of β-methoxysorbic acid was greatly increased, as shown:

NaOMe, mols. %	20	60	100	100	200	400	600
Time (hours)	72	24	0·75	24	96	48	18
Lactone yield, %	—	17	10	5	—	—	—
Acid yield, %	—	7	—	25	44	60	38

The ester (3 g.) was added, in portions, to a solution of sodium methoxide (from 2 g. of sodium) in dry methanol (25 c.c.) with careful cooling. The solution was kept at 0° for 6 hours, then at 20° for 42 hours, the sodium salt of the acid separating as long needles. Water (70 c.c.) was added, the solution was cooled to −10° and acidified with concentrated hydrochloric acid, and the precipitated acid (1·8 g.) was collected.

β-Methoxysorbic acid was also obtained by adding the lactone (300 mg.) to a solution of sodium methoxide (from 0·3 g. of sodium) in methanol (12 c.c.); after standing overnight, the methanol was removed under reduced pressure and the residue dissolved in water. Acidification gave β-methoxysorbic acid (180 mg.) as a crystalline precipitate, m. p. 150° (decomp.). The lactone was prepared in better yield by using the boron trifluoride–mercuric oxide catalyst (method *b*).

(*b*) Boron trifluoride–ether complex (0·3 g.) and mercuric oxide (0·06 g.) were added to dry methanol (4 c.c.). The mixture was heated to 65°, then cooled to 30°. When the ester (2 g.) was added, an exothermic reaction took place, the temperature being kept below 55° by external cooling. The mixture was set aside at 20° for 18 hours, then at 0° for 4 days. The gelatinous precipitate was filtered off and washed with methanol, chloroform (12 c.c.) was added, and the solution was shaken with a saturated solution of potassium hydrogen carbonate (1 c.c.). The dried extract was evaporated under reduced pressure; the residue solidified partly, and was recrystallised from ether. Several crops were obtained; those which had an unsatisfactory m. p. were again recrystallised. The total yield of lactone (m. p. 64—66°) was 1·25 g. The m. p. was not depressed by admixture with a specimen prepared by method (*a*).

Hydrolysis. The lactone (200 mg.) was hydrolysed to ethylideneacetone by heating it under reflux with 20% sulphuric acid, as described for the corresponding diethylamino-compound. Because of the larger scale of the experiment the evolution of carbon dioxide was observed and confirmed by means of barium hydroxide solution.

β-Methoxysorbic acid (100 mg.) was hydrolysed in the same way, except that the reaction was almost instantaneous. In both cases the ketone was isolated as its 2 : 4-dinitrophenylhydrazone, which after crystallisation from ethyl acetate–methanol had m. p. 158—159°, undepressed on admixture with an authentic specimen.

Lactone of 2-Methoxybut-1-en-4-ol-1-carboxylic Acid.—The methyl ester of but-1-yn-4-ol-1-carboxylic acid (2·8 g.) was added to a solution of mercuric oxide (0·05 g.) and boron trifluoride–ether complex (0·3 g.) in dry methanol (6 c.c.). The mixture was warmed to 40° and an exothermic reaction took place; the temperature was then kept below 55°. After cooling and 3 days' standing, isolation of the product with chloroform in the usual way (care being taken to avoid losses due to water-solubility) gave an oil which solidified readily and was pressed on a porous tile at 0° to give crude lactone (1·6 g.), m. p. 66—70°. Digestion with ether, followed by crystallisation from carbon tetrachloride–light petroleum, gave the *lactone* as needles, m. p. 78° (Found: C, 55·95; H, 6·25. $C_6H_8O_3$ requires C, 56·25; H, 6·3%).

Lactone of β-Phenoxy-β-(1-hydroxycyclohexyl)acrylic Acid (II; R = OPh) *and* trans-*β-Phenoxy-β-(1-hydroxycyclohexyl)acrylic Acid and its Methyl Ester.*—The methyl ester of β-(1-hydroxy*cyclo*hexyl)-propiolic acid (4 g.) was added to a suspension of sodium phenoxide (from 0·2 g. of sodium) in phenol (8 g.), and the mixture was heated to 90°; an exothermic reaction took place, the temperature rising to 125°. After cooling and 2 hours' standing at 15°, ether was added and the solution was shaken with 4N-sodium hydroxide solution until free from phenol. The ethereal solution was dried, and the ether evaporated; the residual oil was dissolved in 50% more than the necessary minimum quantity of boiling light petroleum (b. p. 40—60°), seeded with material from a preliminary preparation, and cooled to 15°; the *methyl* ester of the *trans*-acid (2·7 g.) separated in prisms, m. p. 103°, unchanged by recrystallisation from the same solvent (Found: C, 69·55; H, 7·4. $C_{14}H_{20}O_4$ requires C, 69·55; H, 7·3%).

The filtrate was evaporated, and the residual oil was dissolved in methanol (15 c.c.). After removal of a small quantity of flocculent material, water was added until a turbidity just formed; on seeding and cooling to 0° the *lactone* (1·15 g.) separated as lustrous plates, m. p. 69·5°, unchanged on recrystallisation from the same solvent or from light petroleum (Found: C, 73·95; H, 6·4. $C_{13}H_{16}O_3$ requires C, 73·75; H, 6·6%).

By extracting the filtrate with ether, drying, evaporating, and crystallising the residue from light petroleum, a further 0·25 g. of the *trans*-hydroxy-ester, m. p. 101—102°, was obtained.

In an exactly similar experiment in which all quantities were reduced to one-quarter of those stated, the yields of hydroxy-ester and lactone were 0·92 g. and 0·09 g., respectively. No explanation of this anomaly can be offered.

A solution of potassium hydroxide (0·5 g.) in water (1·5 c.c.) was added to a solution of the hydroxy-ester (0·8 g.) in methanol (3·5 c.c.). After standing at 20° for 3 days the mixture was acidified and diluted with water; crystallisation of the precipitate from aqueous methanol gave the trans-*hydroxy-*

acid (0·7 g.) as plates, m. p. 192° (decomp.) (Found: C, 68·65; H, 6·9. $C_{15}H_{18}O_4$ requires C, 68·65; H, 6·95%).

Lactone of β-Benzylthio-β-(1-hydroxycyclohexyl)acrylic Acid (II; R = Ph·CH_2·S) *and* trans-*β-Benzylthio-β-(1-hydroxycyclohexyl)acrylic Acid.*—The methyl ester of β-(1-hydroxycyclohexyl)propiolic acid (1 g.) was added to benzylthiol (1 g.) and triethylamine (0·1 g.); an exothermic reaction took place, the temperature rising to 95°. After the mixtures had stood for 1 hour at 20°, a solution of potassium hydroxide (1 g.) in aqueous methanol (20 c.c.) was added. The mixture was kept for 48 hours at 20° and then extracted with ether after dilution with water. The dried extract gave on evaporation an oil which solidified on cooling; crystallisation from light petroleum (b. p. 60—80°) gave the *lactone* (0·29 g.) as needles, m. p. 94° (Found: C, 69·9; H, 6·3. $C_{16}H_{18}O_2S$ requires C, 70·0; H, 6·6%).

The alkaline solution was acidified and extracted with ether, and the extract thoroughly washed with sodium hydrogen carbonate solution; on acidification of the washings, the trans-*hydroxy-acid* (0·98 g.) separated. Crystallisation from aqueous methanol gave plates, m. p. 151—152° (decomp.) (Found: C, 65·75; H, 7·1. $C_{16}H_{20}O_3S$ requires C, 65·7; H, 6·9%).

Repetition of this experiment on a small scale, using 300 mg. of the ester, yielded 190 mg. of the lactone and 163 mg. of the acid. The reaction temperature was rather lower (*ca.* 65°) in this case.

β-Phenoxycrotonic Acid.—Methyl tetrolate (0·5 g.) was added to a suspension of sodium phenoxide (from 0·05 g. of sodium) in phenol (3 g.) at 80°. No reaction took place, and the mixture was therefore warmed to 120°, whereupon an exothermic reaction began, the temperature rising to 160°. After cooling, water was added and the neutral fraction was isolated by extraction with ether, washing the extract well with sodium hydroxide solution, and drying and evaporating it. The crude methyl ester thus obtained was hydrolysed by adding a solution of potassium hydroxide (1 g.) in methanol (6 c.c.) and water (2 c.c.) and keeping it for 18 hours at 20°. The acidic fraction of the mixture was isolated in the usual way, giving the acid (180 mg.; 17%), m. p. 148—150° (decomp.). After crystallisation from benzene–light petroleum (b. p. 80—100°), *β-phenoxycrotonic acid* formed needles, m. p. 151—153° (slight decomp.) (Found: C, 67·35; H, 5·25. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·65%). The yield could probably have been improved if precautions had been taken to minimise the rapid hydrolysis of the product in acid solution.

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305. *Researches on Acetylenic Compounds. Part XX. Some Reactions of the Acetylenic–Ethylenic Aldehyde,* 3-*Methylpent*-2-*en*-4-*yn*-1-*al.*

By Sir Ian Heilbron, E. R. H. Jones, and Marc Julia.

The preparation of the methylpentenynal (I) has been improved and a general survey of its reactions has been made. These included addition of ammonia and amines, conversion into the acetal, and partial hydrogenation of the latter to the diene-acetal, and oxidation and condensation reactions of the aldehyde group. The preparation of methyl 2 : 2-dichlorovinyl ketone and certain of its Grignard reactions are also described.

In Part VI of this series (Jones and Weedon, *J.*, 1946, 937) the preparation and rearrangement of carbinols derived from methyl 2-chlorovinyl ketone were described, 3-methylpent-2-en-4-yn-1-al (I) being obtained by the action of dilute acids on the carbinol resulting from the condensation of acetylene itself with the ketone. This aldehyde represents a novel type of acetylenic compound, and its ready availability and interesting multi-functionality suggested that a survey of its reactions would be desirable.

Repetition of the earlier work resulted in improvements in the preparation both of the carbinol and of the aldehyde; in the latter case yields of up to 90% were obtained by prolonging the acid isomerisation treatment.

Many examples of the β-addition of amines to the acetylenic bond in αβ-acetylenic carbonyl compounds are now known (cf. Bowden, Braude, Jones, and Weedon, *J.*, 1946, 45). Methylpentenynal reacted readily with diethylamine in ethereal solution, giving an *adduct* (II), m. p. 57·5—58°, in 40% yield. As was expected from an analogous reaction with a similarly constituted ketone (Bowden *et al.*, *loc. cit.*), the amine addition occurred at the acetylenic linkage, light-absorption data (see below) providing conclusive evidence of the extended conjugated

(II)	$\lambda_{max.}$, A.	2360	2580 *	3,970	(III) [1]	$\lambda_{max.}$, A. ...	2270	2420	3,780
	$\varepsilon_{max.}$	5000	4000	59,500		$\varepsilon_{max.}$ ...	3500	3000	46,500

* Inflexion.

[1] Bowden, *et al.*, *loc. cit.*

system (N·[C:C]$_2$·C=O chromophore). The considerable water solubility of the adduct and also its feeble basic and aldehydic properties suggest that the dipolar resonance form ($Et_2\overset{+}{N}$=CH·CH=CMe·CH=CH·O^-) contributes very largely towards the resonance hybrid.

HC≡C·CMe:CH·CHO (I.) Et_2N·CH:CH·CH:CH·CO·CH_3 (III.)

Et_2N·CH:CH·CMe:CH·CHO (II.) [*p*-C_6H_4Me·NH·CH:CH·CMe:CH·CH:N·C_6H_4Me-*p*],HCl (IV.)

In its reactions with primary amines, methylpentenynal (I) behaves strictly as a vinylogue (Fuson, *Chem. Reviews*, 1935, **16**, 1) of propargyl aldehyde (cf. Claisen, *Ber.*, 1903, **36**, 3665). In the reaction between the latter aldehyde or its acetal and aniline, Claisen recorded the formation of a monoadduct which he formulated as HC≡C·CH(OH)·NHPh but which is probably more correctly represented as the anilino-aldehyde, NHPh·CH=CH·CHO. On treatment with aniline hydrochloride, or by treatment of the parent aldehyde with two molecular proportions of the hydrochloride, the hydrochloride of the Schiff's base, [NHPh·CH:CH·CH:NPh],HCl, is produced (cf. for analogous reactions, Reitzenstein and Schwerdt, *J. pr. Chem.*, 1907, **75**, 412; Reitzenstein and Bönitsch, *ibid.*, 1912, **86**, 1; Viguier, *Ann. Chim.*, 1913, **28**, 433; Dieterle and Riester, *Chem. Zentr.*, 1937, I, 3910; 1937, II, 714). With aniline, methylpentenynal gave a yellow monoadduct, m. p. 60—63° (presumably NHPh·CH:CH·CMe:CH·CHO), and this with aniline hydrochloride gave the dark violet hydrochloride of an anilino-anil ([NHPh·CH:CH·CMe:CH·CH:NPh],HCl), m. p. 153°. The former of these two compounds was too unstable and the latter too insoluble and unstable to permit of further purification. With *p*-toluidine hydrochloride, however, a more amenable deep violet *hydrochloride* (IV), m. p. 145°, was obtained and an analogous compound was formed with *p*-aminobenzoic acid. In similar reactions with appropriate heterocyclic nitrogen compounds methylpentenynal yields cyanine-type dyes (Jones and Read, B.P. 616,223).

Treatment with anhydrous ammonia in ether left the aldehyde essentially unchanged, but with alcoholic solutions of aqueous ammonia or ammonium carbonate, (I) gave γ-picoline in 10—15% yields, presumably by internal condensation of the mono-adduct (NH_2·CH:CH·CMe:CH·CHO).

In order to render possible a study of the replacement reactions of the acetylenic hydrogen atom, the preparation of the *acetal* (V) of methylpentenynal was investigated. By using ethyl

(V.) HC≡C·CMe=CH·CH$(OEt)_2$ ⟶ CH_2·CH_2·CH_2·CH(OH)·C≡C·CMe=CH·CH$(OEt)_2$ (VI.)

H_2C=CH·CMe=CH·CH$(OEt)_2$ (VII.)

orthoformate and ammonium nitrate in alcohol, a procedure successfully employed by Fischer and Baer (*Helv. Chim. Acta*, 1935, **18**, 514) to obtain acraldehyde acetal, the acetal was produced in 55% yield. It gave copper and silver derivatives, showed light-absorption properties typical of those of a conjugated en-yne and yielded the derivative of the parent aldehyde on treatment with acid and 2 : 4-dinitrophenylhydrazine.

The ethynyl hydrogen atom of propargyl acetal can be replaced in Grignard reactions (cf. Grard, *Ann. Chim.*, 1930, **13**, 343; Heilbron, Jones, and Koch, *J.*, 1942, 735) and the acetal (V) behaved similarly. With butaldehyde the hydroxy-acetal (VI) was obtained although it could not be distilled without undergoing some decomposition; on treatment with semicarbazide hydrochloride in aqueous methanol, however, the *semicarbazone* of the corresponding hydroxy-aldehyde was formed in good yield.

Semihydrogenation of (V) in the presence of a partially poisoned palladium catalyst yielded the *acetal* of *3-methylpenta-2 : 4-dien-1-al* (VII), showing the expected light-absorption properties, and giving the *semicarbazone* and the *2 : 4-dinitrophenylhydrazone* of the aldehyde on treatment with the appropriate reagent in acid media. As a possible alternative route to the diene-aldehyde the preparation and rearrangement of the vinyl-carbinol (VIII) have been studied; the results of this investigation, as yet incomplete, will be reported later.

(VIII.) H_2C:CH·CMe(OH)·CH:CHCl HC≡C·CMe=CH·CH=CH·COR (IX.)

Oxidation of the aldehyde with chromic acid in aqueous acetone solution gave the corresponding *acid* (XI; R = H) but, rather unexpectedly, the yield and conversion were much less satisfactory than in the case of the oxidation of pent-2-en-4-yn-1-ol to an analogous acid (Heilbron, Jones, and Sondheimer, *J.*, 1947, 1586). Other standard methods of aldehyde → acid conversion were even less successful. The aldehyde can be condensed with ketones in the presence of sodium hydroxide, and with acetophenone and acetone the *ketones* (IX; R = Ph and Me, respectively) were obtained, the former as a crystalline solid, m. p. 86—88°.

As an alternative route to acids of the type (XI), the possibility of effecting conversion of dichlorovinyl carbinols (X) into such acids was explored. *Methyl* 2 : 2-*dichlorovinyl ketone* was readily prepared by condensation of acetyl chloride with vinylidene chloride (cf. Catch, Elliott, Hey, and Jones, *J.*, 1948, 278). Unfortunately, on attempted condensation with sodium acetylide in liquid ammonia, none of the expected carbinol (X; R = H) could be obtained.

(X.) $R{\cdot}C{\equiv}C{\cdot}CMe(OH){\cdot}CH{=}CCl_2$ $R{\cdot}C{\equiv}C{\cdot}CMe{=}CH{\cdot}CO_2H$ (XI.)

Hexynylmagnesium bromide, however, yielded the *carbinol* (X; R = Bu^n); with acetylenedimagnesium bromide a small yield of the *glycol*, m. p. 98°, was obtained, and methylmagnesium bromide gave the *carbinol*, $CMe_2(OH){\cdot}CH{:}CCl_2$. Using conditions (cf. Jones and Weedon, *loc. cit.*) under which chlorovinyl-carbinols were converted smoothly into aldehydes, none of the above dichlorovinyl compounds could be converted into acids or acid derivatives. These results were not entirely unexpected, as the effect of the additional chlorine substituent would be to reduce still further the electron availability at the reacting centre, *i.e.*, the hydroxyl group (cf. Braude and Jones, *J.*, 1946, 122, 128). They are also in line with the observations of Kirrmann *et al.* (*Bull. Soc. chim.*, 1940, [v], **7**, 586; 1948, [v], **15**, 168) that in the systems, $CH_2{:}CR{\cdot}CCl_3 \rightleftharpoons CH_2Cl{\cdot}CR{:}CCl_2$, the equilibria lie almost entirely on the side of the dichlorovinyl compounds.

Experimental.

(Absorption spectra were determined in alcoholic solutions, except where stated otherwise).

1-*Chloro*-3-*methylpent*-1-*en*-4-*yn*-3-*ol* (cf. Jones and Weedon, *loc. cit.*).—The rate of addition of the methyl chlorovinyl ketone to the liquid ammonia solution of sodium acetylide has much influence on the yield. By adding the ethereal solution of the ketone very slowly (200 g. during at least 6 hours) and employing an acetylide : ketone ratio of 2·7 : 1, yields of up to 55% have been obtained in fairly large-scale experiments. In the presence of a little quinol and in an atmosphere of nitrogen the carbinol can be kept at 0° without appreciable alteration for several months.

3-*Methylpent*-2-*en*-4-*yn*-1-*al* (I) (cf. Jones and Weedon, *loc. cit.*).—1-Chloro-3-methylpent-1-en-4-yn-3-ol (30 g.) was added to 2N-sulphuric acid (2500 c.c.). A trace of quinol was added, the air displaced by nitrogen, and the mixture kept in the dark at room temperature (about 15°). After about one day the carbinol dissolved; after two days an oil began to separate and the liquid, originally very pale yellow, turned orange. After four days the solution was saturated with ammonium sulphate and extracted three times with ether. In some experiments, when the room temperature was 25°, the rearrangement was completed after two days. After addition of a trace of quinol, the ethereal solution was dried and evaporated, and the residue fractionated in nitrogen, giving the aldehyde as a very pale yellow liquid (19 g.; 90%), b. p. 45°/12 mm.; $n_D^{21°}$ 1·5018 (Jones and Weedon, *loc. cit.*, give b. p. 53—54°/20 mm.; $n_D^{23°}$ 1·4993).

5-*Diethylamino*-3-*methylpenta*-2 : 4-*dien*-1-*al* (II).—A solution of diethylamine (1·5 g.) in ether (10 c.c.) was slowly added to a solution of methylpentenynal (1·9 g.) in ether (10 c.c.) with ice cooling in an atmosphere of nitrogen. The mixture was kept for 2 hours at room temperature and 12 hours at 0° in the dark. The solution, separated from a little dark oil that had been deposited, was dried and evaporated. Distillation of the residue gave (i) dark red oil (0·5 g.), b. p. 105—110°/0·05 mm., and (ii) red oil (0·8 g.), b. p. 110—111°/0·05 mm. Fraction (ii) crystallised on cooling, and (i) crystallised after seeding. Recrystallisation of the combined material from ether–light petroleum (b. p. 40—60°) in the cold in nitrogen gave the *diethylamino-aldehyde* as golden-yellow leaflets, m. p. 57·5—58° (Found: C, 71·45; H, 9·55; N, 8·25. $C_{10}H_{17}ON$ requires C, 71·8; H, 10·25; N, 8·35%).

Hydrochloride of 1-p-*Toluidino*-5-p-*tolylimino*-3-*methylpenta*-1 : 3-*diene* (IV).—A solution of methylpentenynal (1 g.) in methanol (5 c.c.) was added slowly to *p*-toluidine (2·2 g.) in methanol (10 c.c.) and 2N-hydrochloric acid (10 c.c.), under nitrogen, the temperature being kept below 20°. The mixture turned deep violet and on standing for 3 hours at 0° crystals were deposited which after recrystallisation from methanol gave the *hydrochloride* (1·2 g.) as fine deep violet needles, m. p. 145° (Found: C, 73·5; H, 7·3; N, 8·75. $C_{20}H_{23}N_2Cl$ requires C, 73·5; H, 7·1; N, 8·55%). Light absorption: Maximum, 5250 A.; ε = 41,000.

Hydrochloride of 1-p-*Carboxyanilino*-5-p-*carboxyanilo*-3-*methylpenta*-1 : 3-*diene*.—A solution of methylpentenynal (5 g.) in methanol (10 c.c.) was slowly added to a solution of *p*-aminobenzoic acid (15 g.), methanol (40 c.c.), and 2N-hydrochloric acid (25 c.c.) under nitrogen, the temperature being kept below 20°. The mixture turned deep violet and a solid separated which was only partly soluble in boiling methanol. On cooling the solution, crystals (0·7 g.) were deposited which on recrystallisation from methanol gave the *hydrochloride* as reddish-violet prisms, m. p. indefinite, decomposition above 180—200° (Found: N, 6·8. $C_{20}H_{19}O_4N_2Cl$ requires N, 7·25%). Light absorption: Maxima, 2280, 4300, and 5250 A.; ε = 22,500, 7000, and 9500, respectively. The deep red alcoholic solution of the compound lost its colour after a few hours at room temperature, which explains the low intensity of the long-wave maximum.

γ-Picoline from Methylpentenynal.—(*a*) Methylpentenynal (1 g.) was added to concentrated aqueous ammonia (50 c.c.) and the mixture was made homogeneous by addition of alcohol. After 12 hours, water (100 c.c.) was added and the product was isolated with ether. Distillation at 100 mm. gave an oil (0·1 g.) which yielded γ-picoline picrate, m. p. 167°, undepressed on admixture with an authentic specimen.

(*b*) (Cf. Bowden and Jones, *J.*, 1946, 953.)—Ammonium carbonate (1 g.) was dissolved in the minimum amount of water, and alcohol (3 c.c.) was added; this solution was added to a solution of

methylpentenynal (1 g.) in alcohol (3 c.c.) and the mixture was heated under reflux for ½ hour. Isolation with ether and distillation at 100 mm. gave an oil (0·15 g.) which yielded a picrate identical with that obtained in (*a*).

Acetal of 3-*Methylpent*-2-*en*-4-*yn*-1-*al* (V).—To a mixture of methylpentenynal (3 g.) and ethyl orthoformate (4 g.) was added a hot solution of ammonium nitrate (0·2 g.) in alcohol (8 c.c.) and the mixture was heated under reflux for 5 minutes. After cooling, ether (20 c.c.) was added and the mixture was shaken repeatedly with dilute aqueous ammonia until no more amorphous material separated. After drying and evaporation, distillation under reduced pressure gave a pale yellow oil (3·6 g.). This was shaken with concentrated aqueous sodium hydrogen sulphite solution, the residual oil was isolated with ether, and distillation gave 1 : 1-*diethoxy*-3-*methylpent*-2-*en*-4-*yne* (2·8 g.), b. p. 77°/12 mm., $n_D^{18°}$ 1·4500 (Found: C, 71·25; H, 9·55. $C_{10}H_{18}O_2$ requires C, 71·4; H, 9·6%). Light absorption: Maximum, 2230 A.; ε = 12,000. The acetal gave a white silver salt with ammoniacal silver nitrate. Steam distillation in the presence of 2N-sulphuric acid gave an oil, and thence a 2 : 4-dinitrophenylhydrazone, m. p. 144°, undepressed on admixture with an authentic specimen of methylpentenynal 2 : 4-dinitrophenylhydrazone, m. p. 144—145° (Jones and Weedon, *loc. cit.*), was prepared.

Condensation of the Acetal with Butaldehyde.—Ethylmagnesium bromide (from magnesium, 0·9 g.) was prepared in ether (50 c.c.) and the ether was evaporated and replaced by dry benzene (50 c.c.). This solution was dropped into a solution of 1 : 1-diethoxy-3-methylpent-2-en-4-yne (6 g.) in dry benzene (40 c.c.) in an atmosphere of nitrogen with stirring and ice cooling during 1 hour. After a further 3 hours' stirring at 0°, butaldehyde (3 g.) in dry benzene (30 c.c.) was slowly added and stirring was continued for a further 4 hours at room temperature. The Grignard complex was decomposed with an ice-cold solution of ammonium chloride (20 g.) in water (100 c.c.), the benzene layer separated, and the aqueous layer extracted with benzene. The combined benzene solutions were washed and dried and evaporated under reduced pressure. Fractionation of the residue gave (i) recovered acetal (3 g.), b. p. 80°/0·05 mm., $n_D^{20°}$ 1·4460, and (ii) crude condensation product (3 g.), b. p. 102—104°/0·01 mm., $n_D^{20°}$ 1·4649. The latter could not be fractionated without some decomposition; consequently, a sample (200 mg.) was gently heated with a solution of semicarbazide hydrochloride (150 mg.) in aqueous methanol. Crystals (200 mg.; 80%) separated on cooling, and recrystallisation from alcohol gave the *semicarbazone* of 3-*methylnon*-2-*en*-4-*yn*-6-*ol*-1-*al* as needles, m. p. 197—198° (Found: N, 19·0. $C_{11}H_{17}O_2N_3$ requires N, 18·8%). Light absorption: Maximum, 2940 A.; ε = 31,000; inflexion, 3040 A.; ε = 27,000.

Acetal (VII), *Semicarbazone, and* 2 : 4-*Dinitrophenylhydrazone of* 3-*Methylpenta*-2 : 4-*dien*-1-*al*.—1 : 1-Diethoxy-3-methylpent-2-en-4-yne (9 g.) in methyl acetate (20 c.c.) was shaken in an atmosphere of hydrogen in the presence of a partially poisoned palladium catalyst (1 g.; Isler, Huber, Ronco, and Kofler, *Helv. Chim. Acta*, 1947, **30**, 1911). The hydrogenation was interrupted when the required amount of hydrogen had been absorbed (1280 c.c., corresponding to 1 mol. of hydrogen). The catalyst and solvent were removed and the residue was shaken with concentrated aqueous sodium hydrogen sulphite and extracted with ether. After the ethereal solution had been washed with water, dried, and evaporated, fractionation of the residue gave the *diene acetal* (5·5 g.), b. p. 79—80°/18 mm., $n_D^{23°}$ 1·4550 (Found: C, 69·7; H, 10·6. $C_{10}H_{18}O_2$ requires C, 70·55; H, 10·65%). Light absorption: Maximum, 2230 A.; ε = 20,500; inflexion, 2280 A.; ε = 18,500.

The diene acetal easily gave the 2 : 4-*dinitrophenylhydrazone*, which separated from ethyl acetate–methanol as deep purple needles, m. p. 131—132° (Found: N, 19·9. $C_{12}H_{12}O_4N_4$ requires N, 20·3%). Light absorption in chloroform: Maxima, 2600, 3029, and 3900 A.; ε = 12,500, 10,000, and 30,000, respectively. On treatment of the acetal with semicarbazide hydrochloride in aqueous methanol, the *semicarbazone* was obtained as needles from ethyl acetate, m. p. 166—167° (Found: N, 27·5. $C_7H_{11}ON_3$ requires N, 27·45%). Light absorption: Maximum, 2940 A.; ε = 38,000.

2-*Methylbut*-1-*en*-3-*yne*-1-*carboxylic Acid* (XI; R = H).—A solution of chromium trioxide (5 g.) and concentrated sulphuric acid (8 g.) in water (20 c.c.) was added during an hour to a stirred solution of methylpentenynal (6 g.) in acetone (45 c.c.) in an atmosphere of nitrogen, the temperature being kept below 25°. The mixture was allowed to stand with occasional stirring for 2 hours; it was then diluted with water, saturated with potassium chloride, and thoroughly extracted with ether. The combined ethereal extracts were shaken four times with 2N-sodium hydrogen carbonate solution (90 c.c. in all), the extracts were acidified with 2N-sulphuric acid (100 c.c.) and saturated with potassium chloride, and the acidic material was isolated by thorough extraction with ether. Evaporation gave an orange-yellow oil (1·0 g.) which crystallised partially on trituration with light petroleum (b. p. 60—80°). Recrystallisation from this solvent (charcoal) gave 2-*methylbut*-1-*en*-3-*yne*-1-*carboxylic acid* (200 mg.) as flat needles, m. p. 107·5—109° (Found: C, 65·2; H, 5·5. $C_6H_6O_2$ requires C, 65·45; H, 5·5%). Light absorption: Maximum, 2480 A.; ε = 16,000. Some 3·2 g. of aldehyde were recovered from the neutral portion of the product.

1-*Benzoyl*-4-*methylhexa*-1 : 3-*dien*-5-*yne* (IX; R = Ph).—Methylpentenynal (0·5 g.) and acetophenone (0·6 g.) were mixed under nitrogen at room temperature and sodium hydroxide (0·2 g.), dissolved in water (2 c.c.) and alcohol (1 c.c.), was added, the temperature being kept below 20° by external cooling. The mixture became very dark and after 15 minutes an excess (3 c.c.) of 2N-sulphuric acid was added. A dark red oil separated which crystallised partly when kept overnight at 0°. The crystals (200 mg.) m. p. 82—85°, were freed from oil on a porous plate and several recrystallisations from aqueous methanol gave the *ketone* as dark yellow flat needles, m. p. 86—88° (Found: C, 85·5; H, 6·05. $C_{14}H_{12}O$ requires C, 85·7; H, 6·15%). Light absorption in methanol: Maxima, 2280 and 3240 A.; ε = 10,000 and 32,000, respectively.

6-*Methylocta*-3 : 5-*dien*-7-*yn*-2-*one* (1-*Acetyl*-4-*methylhexa*-1 : 3-*dien*-5-*yne*) (IX; R = Me).—Methylpentenynal (3 g.) was dissolved in acetone (6 c.c.) and the solution was cooled in ice in an atmosphere of nitrogen. Sodium hydroxide (0·8 g.), dissolved in water (3 c.c.) and alcohol (3 c.c.), was added; the solution darkened considerably and after ½ hour acidification with 2N-sulphuric acid precipitated a dark red oil which was taken up in ether. The ethereal solution was washed and dried, and after evaporation at room temperature under reduced pressure, the residue was distilled, giving the *ketone* (1·5 g.; 30%)

as a very pale yellow oil, b. p. 44—46°/10^{-3} mm., $n_D^{18°}$ 1·5630. This oil solidified on keeping at 0° and melted a little above room temperature (Found: C, 80·2; H, 7·45. $C_9H_{10}O$ requires C, 80·55; H, 7·5%). Light absorption: Maximum, 2390 A.; $\varepsilon = 12{,}500$. At the end of the distillation, the residue sometimes exploded if heated above *ca.* 75°. The *semicarbazone* separated from alcohol as very pale yellow needles. It decomposed above about 185° without melting sharply (Found: N, 21·95. $C_{10}H_{13}ON_3$ requires N, 22·0%). Light absorption: Maxima, 3110 and 3260 A.; $\varepsilon = 51{,}500$ and 45,000, respectively.

6-*Methyloctan*-2-*one*.—A solution of the above ketone (0·85 g.) in alcohol (50 c.c.) was shaken in an atmosphere of hydrogen in presence of platinic oxide catalyst (20 mg.), 610 c.c. of hydrogen being absorbed in a little more than ½ hour (theoretical amount: 565 c.c.). The catalyst was filtered off, and the solvent removed under reduced pressure: the remaining oil was distilled, giving 6-*methyloctan*-2-*one* (0·6 g.) as a pleasant-smelling liquid, b. p. 70°/10 mm., $n_D^{18°}$ 1·4250 (Found: C, 76·25; H, 13·05. $C_9H_{18}O$ requires C, 76·0; H, 12·75%). The *semicarbazone* formed leaflets from aqueous alcohol, m. p. 131° (Found: N, 20·9. $C_{10}H_{21}ON_3$ requires N, 21·1%).

Methyl 2 : 2-*Dichlorovinyl Ketone*.—Acetyl chloride (50 g.) was cooled in a freezing mixture and finely powdered aluminium chloride (50 g.) was slowly added with vigorous stirring. The freezing mixture was then replaced by a water-bath at room temperature, and vinylidene chloride (30 g.) was slowly dropped in during 1½ hours. At first the mixture became less viscous but the viscosity subsequently increased considerably. After being stirred for a further ½ hour the reaction mixture was poured on crushed ice (600 g.); a heavy oil separated which soon crystallised in long white needles, melting at about room temperature. This product was taken up in carbon tetrachloride and the aqueous solution was extracted several times with the same solvent. The combined solutions (250 c.c.) were washed twice with water, and then hot water (250 c.c.) was added and steam was passed through. The distilled organic layer was separated and the aqueous phase was extracted twice with carbon tetrachloride.

The combined tetrachloride solutions were washed with water and dried and evaporated (after the addition of traces of quinol and magnesium oxide) under reduced pressure below 30° through a Dufton column (8″). The residual ketone was then rapidly distilled under reduced pressure and subsequent fractionation gave *methyl* 2 : 2-*dichlorovinyl ketone* (32·5 g.; 80%), b. p. 45°/10 mm., 58°/15 mm., $n_D^{20°}$ 1·4940 (Found: C, 34·6; H, 2·8. $C_4H_4OCl_2$ requires C, 34·55; H, 2·9%). Light absorption: Maximum, 2390—2420 A.; $\varepsilon = 12{,}000$. As with methyl chlorovinyl ketone, the immediate fractionation of the crude product was found to be essential. The 2 : 4-*dinitrophenylhydrazone* crystallised from benzene in orange-red prisms, m. p. 195° (Found: N, 17·2. $C_{10}H_8O_4N_4Cl_2$ requires N, 17·55%). Light absorption in chloroform: Maximum, 3740 A.; $\varepsilon = 30{,}000$.

1 : 1-*Dichloro*-3-*methylnon*-1-*en*-4-*yn*-3-*ol*.—Ethylmagnesium bromide was prepared from magnesium (2·1 g.) in ether. Hex-1-yne (9 g.), dissolved in dry ether (100 c.c.), was added during 2 hours with ice cooling, and the mixture was then heated under reflux for 3 hours. Methyl 2 : 2-dichlorovinyl ketone (10 g.) in dry ether (100 c.c.) was added slowly with cooling; the Grignard complex separated as a white suspension and the mixture was set aside overnight. The complex was decomposed with an ice-cold solution of ammonium chloride (27 g.) in water (100 c.c.) and the product was isolated in the usual manner. Distillation gave 1 : 1-*dichloro*-3-*methylnon*-1-*en*-4-*yn*-3-*ol* (13·5 g.; 85%), b. p. 90°/0·5 mm., 65°/0·05 mm., $n_D^{20°}$ 1·4909 (Found: C, 54·45; H, 6·6. $C_{10}H_{14}OCl_2$ requires C, 54·3; H, 6·4%). The carbinol showed no absorption of appreciable intensity in the range 2200—4000 A.

1 : 1-*Dichloro*-3-*methylbut*-1-*en*-3-*ol*.—Methylmagnesium bromide was prepared from magnesium (1·8 g.) in ether, and methyl 2 : 2-dichlorovinyl ketone (10 g.) in ether (100 c.c.) was dropped in with ice cooling. The mixture was stirred for 4 hours at room temperature and the complex which had separated was decomposed with an ice-cold solution of ammonium chloride (22 g.) in water (100 c.c.). The product was isolated with ether and distillation gave 1 : 1-*dichloro*-3-*methylbut*-1-*en*-3-*ol* (5·8 g.; 55%), b. p. 63°/13 mm., $n_D^{19°}$ 1·4814 (Found: C, 37·8; H, 4·9; Cl, 44·35. $C_5H_8OCl_2$ requires C, 38·7; H, 5·2; Cl, 45·7%). The p-*nitrobenzoate* crystallised from alcohol as very pale yellow leaflets, m. p. 97—98° (Found: N, 4·85. $C_{12}H_{11}O_4NCl_2$ requires N, 4·6%).

1 : 1 : 8 : 8-*Tetrachloro*-3 : 6-*dimethylocta*-1 : 7-*dien*-4-*yn*-3 : 6-*diol*.—Ethylmagnesium bromide was prepared from magnesium (2·4 g.) and ethyl bromide (11 g.) in ether (100 c.c.). The ether was then distilled off and at the same time replaced by dry benzene (200 c.c.). Distillation was continued until the temperature of the vapours reached 70°. The reaction flask was then cooled and a fairly rapid stream of acetylene was passed in for 4 hours with ice cooling. Acetylenedimagnesium bromide separated as a white crystalline powder. Methyl 2 : 2-dichlorovinyl ketone (14 g.) in dry benzene (75 c.c.) was then added during ½ hour with cooling. The mixture was stirred at room temperature for a further 3 hours and the Grignard complex was decomposed with an ice-cold solution of ammonium chloride (30 g.) in water (150 c.c.). The product (13 g.) was isolated *via* benzene, the solution being treated with charcoal, and partly crystallised on standing at 0°. The crystals (2 g.; 15%) were washed free from oil with light petroleum (b. p. 40—60°) and crystallisation from light petroleum (b. p. 60—80°) gave the *diol* (1·5 g.) as long fine needles, m. p. 98° (Found: C, 40·0; H, 3·25. $C_{10}H_{10}O_2Cl_4$ requires C, 39·5; H, 3·3%).

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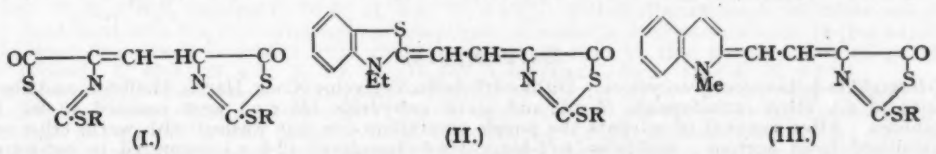
306. *Studies in the Azole Series. Part XV. The Reactivity of 4-Ethoxymethylene-5-thiazolones and -oxazolones.**

By A. H. Cook, G. Harris, and G. Shaw.

Various *S*-substituted 2-mercapto-4-ethoxymethylenethiazolones have been condensed with compounds containing reactive methyl or methylene groups to give stable pigments (*e.g.*, II, III, VII). Analogous 4-ethoxymethyleneoxazolones undergo similar condensations to give pigments such as (VIII) and (IX).

Part IV of this series (*J.*, 1948, 1056) included a description of the preparation of 2-ethylthio-5-thiazolone and thence of its 4-ethoxymethylene derivative. It has now been found that these two compounds easily interact to give *methinbis*-4-[2-(*ethylthio-5-thiazolone*)] (I; R = Et), a compound which is more conveniently prepared by condensing 2 moles of 2-ethylthio-5-thiazolone with one of ethyl orthoformate. The analogous *benzylthio*-compound



(I; R = CH_2Ph) was prepared from 2-benzylthio-5-thiazolone and its 4-ethoxymethylene derivative.

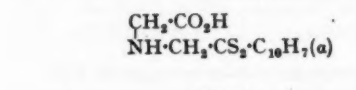
The ethoxymethylene compounds mentioned above reacted rapidly with other compounds containing reactive methyl or methylene groups and the *dimethin dyes* (II and III; R = Et or CH_2Ph) were prepared from 2-methylbenzthiazole ethiodide and quinaldine methiodide, respectively; these preparations were notably facile when carried out in cold ethanol in presence of triethylamine, the products crystallising within a few minutes. Like the other dyes described below, these were surprisingly stable; for example, (II and III; R = CH_2Ph) could be boiled with concentrated sulphuric acid without suffering apparent change apart from being converted into water-soluble sulphonates. The dyes also gave water-soluble products when heated with methyl sulphate but the detailed chemistry of these reactions was not studied. This stability suggested the preparation of more readily substituted dyes, and α-chloromethylnaphthalene, glycine, and carbon disulphide were allowed to interact to give *α-naphthyl N-carboxymethyldithioacetate* (IV) which was cyclised in presence of ethyl orthoformate, and the ethoxymethylene compound condensed with the second components to give the *dyes* (II and III; R = $CH_2{\cdot}C_{10}H_7$-α). Quite similarly, glycine, carbon disulphide, and ethyl chloroformate gave *N*-carbethoxydithiocarboxyglycine (V) (CPS 583; Cook, Harris, and Heilbron, *J.*, 1948, 1056) and thence 2-*carbethoxythio*-4-*ethoxymethylene*-5-*thiazolone* (VI), which afforded the dyes (II and III; R = CO_2Et). Compound (VI) condensed also with facility with 1 : 3 : 3-trimethyl-2-methyleneindoline to give the *dye* (VII). The products (II and III; R = CO_2Et) have additional interest as dyes in that they are readily hydrolysed by cold alkali, the products suffering decarboxylation on acidification and being precipitated still in pigmented form.

These experiments suggested that ethoxymethyleneoxazolones might be similarly useful. 2-Phenyl-, 2-*n*-amyl-, and 2-benzyl-4-ethoxymethyleneoxazolones all condensed at once in cold methanol with quinaldine or 2-methylbenzthiazole quaternary salts and the dyes (VIII) and (IX) were isolated.

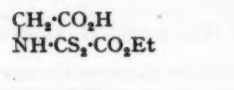
All these dyes were remarkable for their intense colour in spite of the simple nature of the thiazolone and oxazolone rings. That the bathochromic influence is due to these ring systems is shown by the fact that the absorption spectra were not markedly different in the two series of dyes, and in the thiazolone series were not noticeably dependent on the radical attached to the mercapto-residue. It is remarkable that the instability of the oxazolone or thiazolone ring seems to be almost completely suppressed in these dyes: the mercaptothiazolone dyes, for example, reacted with bases (piperidine) to give water-soluble products which still retained dye character and presumably still contained the two terminal heterocyclic rings, though the chemistry of these modified dyes remains to be examined. The colour of all the dyes described in this paper was changed by alkali, but the original colour returned

* This work was carried out in 1945 and an account of it was submitted as CPS 584 for the information of the Committee for Penicillin Syntheses, and for publication as soon as permissible.

on neutralisation or acidification. The dyes were reduced in alcoholic solution to colourless leuco-compounds (*e.g.*, by sodium dithionite) which were reoxidised by air to the original dyes.



(IV.)



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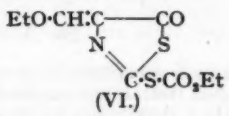

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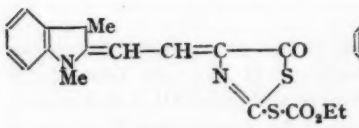
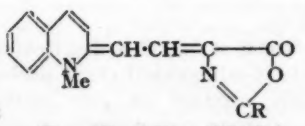
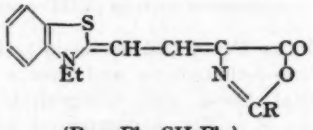


(VII.)



(R = Ph, C_5H_{11}, CH_2Ph.)
(VIII.)



(R = Ph, CH_2Ph.)
(IX.)

Experimental.

2-Benzylthio-5-thiazolone Derivatives.—Dithiocarbobenzoxyglycine (Cook, Harris, Heilbron, and Shaw, *loc. cit.*) (5 g.), ethyl orthoformate (5 g.), and acetic anhydride (25 c.c.) were warmed to 90° for 30 minutes. After removal of solvents the purple crystalline dye was washed with warm ether and recrystallised from acetone; *methinbis*-[4-(*2-benzylthio-5-thiazolone*)] (2·5 g.) separated in red-purple needles, m. p. 187° (decomp.) (Found: C, 55·2; H, 3·7; N, 6·2. $C_{21}H_{16}O_2N_2S_4$ requires C, 55·2; H, 3·5; N, 6·1%). 2-Benzylthio-4-ethoxymethylene-5-thiazolone (0·17 g.) and quinaldine methiodide (0·21 g.) in ethanol (10 c.c.) were treated with triethylamine (0·1 c.c.). The solution immediately became deep violet and on standing the dye (0·3 g.) crystallised. *Dimethin*[2-(*1-methylquinoline*)][4-(*2-benzylthio-5-thiazolone*)] recrystallised from ethanol in dark violet needles with a green sheen, m. p. 210° (decomp.) (Found: C, 67·7; H, 4·64; N, 7·6. $C_{32}H_{16}ON_2S_2$ requires C, 67·7; H, 4·65; N, 7·2%). 2-Benzyl-4-ethoxymethylene-5-thiazolone (0·65 g.) and 2-methylbenzthiazole ethiodide (0·77 g.) in warm methanol (20 c.c.) were treated with triethylamine (1 c.c.), and a mass of dark red needles was soon deposited. *Dimethin*[2-(*1-ethylbenzthiazole*][4-(*2-benzylthio-5-thiazolone*)] (yield, 1·15 g.) crystallised from aqueous acetone in needles, m. p. 175° (Found: C, 61·25; H, 4·6. $C_{21}H_{16}ON_2S_2$ requires C, 61·4; H, 4·4%).

2-Ethylthio-5-thiazolone Derivatives.—2-Ethylthio-4-ethoxymethylene-5-thiazolone was condensed with quinaldine methiodide and with 2-methylbenzthiazole ethiodide as in previous similar reactions in presence of triethylamine. *Dimethin*[2-(*1-methylquinoline*)][4-(*2-ethylthio-5-thiazolone*)] crystallised from ethanol–water in purple or almost black needles with a green lustre, m. p. 172° (decomp.) (Found: C, 62·2; H, 4·8; N, 8·9. $C_{17}H_{16}ON_2S_2$ requires C, 62·2; H, 4·9; N, 8·5%). *Dimethin*[2-(*1-methylbenzthiazole*)][4-(*2-ethylthio-5-thiazolone*)] separated from ethanol in needles, m. p. 173—174° (Found: C, 55·3; H, 4·5; N, 8·4. $C_{16}H_{16}ON_2S_3$ requires C, 55·2; H, 4·6; N, 8·0%). The ethoxymethylene compound (2·7 g.) and 2-ethylthio-5-thiazolone (2 g.) in methanol (5 c.c.) were treated with triethylamine (4 drops), and the solution boiled. On cooling the deep red solution, the dye crystallised. *Methinbis*[4-(*2-ethylthio-5-thiazolone*)] crystallised from ethanol in glistening violet laths, m. p. 150° (Found: C, 39·7; H, 3·8; N, 8·6. $C_{11}H_{12}O_2N_2S_4$ requires C, 39·8; H, 3·6; N, 8·4%). The same compound was obtained in small yield by boiling the ethoxymethylene compound with alcoholic hydrochloric acid (Found: C, 40·2; H, 3·7%).

2-Ethylthio-4-ethoxymethylene-5-thiazolone (0·22 g.) and 8-amino-6-methoxyquinoline (0·17 g.) were mixed in ethanol (3 c.c.). On scratching, the condensation product (0·35 g.) separated as an orange-yellow precipitate. *2-Ethylthio-4-(6′-methoxy-8′-quinolylaminomethylene)-5-thiazolone* crystallised from ethanol in clusters of yellow needles, m. p. 167—168° (Found: C, 55·3; H, 4·4; N, 12·0. $C_{16}H_{15}O_2N_3S_2$ requires C, 55·6; H, 4·4; N, 12·15%). It was only slightly soluble in dilute mineral acids. The same quinoline was similarly condensed with 2-benzylthio-4-ethoxymethylene-5-thiazolone. *2-Benzylthio-4-(6′-methoxy-8′-quinolylaminomethylene)-5-thiazolone* crystallised from much ethanol in yellow needles, m. p. 159° (Found: C, 61·5; H, 4·3; N, 10·4. $C_{21}H_{17}O_2N_3S_2$ requires C, 61·9; H, 4·2; N, 10·3%).

2-Benzyl-5-oxazolone Derivative.—2-Benzyl-4-ethoxymethyleneoxazolone (0·6 g.) and quinaldine methiodide (0·93 g.) in methanol (12 c.c.) were treated with triethylamine (0·1 g.). Diluting the magenta solution with a little water and keeping it at 0° gives the crystalline dye. It was soluble in most organic solvents but crystallised from aqueous pyridine in long needles with a green lustre. *Dimethin*-[2-(*1-methylquinoline*)][4-(*2-benzyl-5-oxazolone*)] had m. p. 184—185° (Found: N, 8·2. $C_{32}H_{16}O_2N_2$ requires N, 8·2%). Light absorption (ethanol): max. at $\lambda = 5100$ A., $\varepsilon = 53{,}500$. 2-Amyl-4-ethoxymethyleneoxazolone was condensed similarly. *Dimethin*[2-(*1-methylquinoline*)][4-(*2-amyl-5-oxazolone*)] separated from aqueous pyridine in clusters of fine purple needles, m. p. 109—110° (Found: C, 74·3; H, 6·9; N, 8·8. $C_{20}H_{22}O_2N_2$ requires C, 74·5; H, 6·8; N, 8·7%). Light absorption (ethanol): max. at $\lambda = 5140$ A., $\varepsilon = 73{,}500$.

2-Phenyl-5-oxazolone Derivatives.—2-Methylbenzthiazole ethiodide (0·22 g.) and 2-phenyl-4-ethoxymethyleneoxazolone (0·2 g.) in methanol (7 c.c.) were treated with triethylamine (3 drops). A deep red colour appeared within 10 seconds and the reaction mixture soon set to a mass of deep red needles (0·2 g.). *Dimethin*[2-(*1-ethylbenzthiazole*)][4-(*2-phenyl-5-oxazolone*)] separated from ethanol in red laths (occasionally in plates), m. p. 210° (Found: C, 68·7; H, 4·6; N, 6·1. $C_{20}H_{16}O_2N_2S$ requires C, 69·0; H, 4·6; N, 8·0%). Light absorption (acetone): max. at $\lambda\lambda = 5100$, 4850 A. Quinaldine methiodide

was used similarly, and *dimethin*[2-(1-*methylquinoline*)][4-(2-*phenyl*-5-*oxazolone*)] separated from ethanol, or better from pyridine containing a little water, in almost black permanganate-like needles, m. p. 222° (Found: C, 76·4; H, 4·8. $C_{21}H_{16}O_2N_2$ requires C, 76·8; H, 4·9%). Light absorption (acetone): max. at λλ = 5310, 4980 A.

2-α-*Naphthylmethylthio*-5-*thiazolone Derivatives.*—Glycine (10 g.) in water (15 c.c.) was treated with an ice-cold solution of potassium hydroxide (15·5 g.) in water (20 c.c.) and shaken for 3—4 hours with carbon disulphide (10·1 g.). The clear solution was then further shaken with 1-chloromethylnaphthalene (23·5 g.) for 40 hours. The solid was filtered off and shaken with 2N-hydrochloric acid (250 c.c.), and the liberated acid (31 g.) soon solidified. *α-Naphthyl N-carboxymethyldithioacetate* crystallised from chloroform–light petroleum or from chlorobenzene in needles, m. p. 196—198° (Found: C, 58·1; H, 4·2; N, 4·3. $C_{14}H_{13}O_2NS_2$ requires C, 57·7; H, 4·5; N, 4·8%). The acid (3·8 g.), ethyl orthoformate (7·0 g.), and acetic anhydride (6 c.c.) were heated together for 30 minutes on the steam-bath, the solution evaporated in a vacuum and the crude oil used directly. The crude ethoxymethylene compound (1·7 g.) was dissolved in boiling ethanol (70 c.c.), and quinaldine methiodide (1·5 g.) and triethylamine (1 c.c.) added. The dye commenced to crystallise immediately. It was practically insoluble in ethanol and was thoroughly extracted with that solvent and recrystallised from chloroform–ether. *Dimethin*[2-(1-*methylquinoline*)][4-(2-α-*naphthylmethylthio*-5-*thiazolone*)] separated in thin purple laths which had a green lustre, m. p. 216° (decomp.) (yield, 70%) (Found: C, 70·7; H, 4·5; N, 6·4. $C_{26}H_{20}ON_2S_2$ requires C, 70·9; H, 4·6; N, 6·4%). 2-Methylbenzthiazole ethiodide was similarly condensed with the ethoxymethylene compound; *dimethin*[2-(1-*ethylbenzthiazole*)][4-(2-α-*naphthylmethylthio*-5-*thiazolone*)] separated from glycol monomethyl ether in thin purple laths, m. p. 188—189° (Found: C, 65·3; H, 4·3; N, 5·9. $C_{24}H_{20}ON_2S_3$ requires C, 65·2; H, 4·4; N, 6·1%).

2-*Carbethoxythio*-5-*thiazolone Derivatives.*—Glycine (5 g.) in water (6 c.c.) was treated, with strong cooling, with potassium hydroxide (7·7 g.) in water (10 c.c.), and the solution shaken with carbon disulphide (5·1 g.) until a clear brown solution was obtained. Ethyl chloroformate (7·2 g.) was added, and the mixture again shaken, with cooling. A vigorous reaction set in and much solid separated. Ice and water were added to effect solution, and acidification then precipitated the produot (yield, 59%). *N*-Carbethoxydithiocarboxyglycine crystallised from ether–light petroleum in rhombcidal prisms, m. p. 125° (Found: C, 32·6; H, 4·0; N, 6·3. Calc. for $C_6H_9O_4NS_2$: C, 32·3; H, 4·0; N, 6·5%). The preceding compound (5 g.), ethyl orthoformate (7 c.c.), and acetic anhydride (40 c.c.) were heated to 90° for 25 minutes, and the solution evaporated in a vacuum. 2-*Carbethoxythio*-4-*ethoxymethylene*-5-*thiazolone* crystallised from light petroleum in needles, m. p. 83° (Found: C, 41·1; H, 4·4. $C_9H_{11}O_4NS_2$ requires C, 41·4; H, 4·2%). The ethoxymethylene compound (0·15 g.) was condensed with 2-methylbenzthiazole ethiodide (0·13 g.) in methanol (5 c.c.) containing triethylamine (3 drops). The deep red solution soon deposited crystals of the pure dye (0·22 g.), m. p. 174—175°. *Dimethin*[2-(1-*ethylbenzthiazole*)][4-(2-*carbethoxythio*-5-*thiazolone*)] crystallised from methanol in red laths, m. p. 174—175° (Found: C, 52·0; H, 4·1; N, 7·3. $C_{17}H_{16}O_3N_2S_3$ requires C, 52·0; H, 4·1; N, 7·1%). Condensation with quinaldine methiodide was carried out similarly in excellent yield, *dimethin*[2-(1-*methylquinoline*)-[4-(2-*carbethoxythio*-5-*thiazolone*)] crystallising from methanol in deep violet needles or laths, m. p. 168—169° (decomp.) (Found: C, 57·9; H, 4·3. $C_{16}H_{16}O_3N_2S_2$ requires C, 58·0; H, 4·3%). The ethoxymethylene compound (0·13 g.) was mixed with 1 : 3 : 3-trimethyl-2-methyleneindoline (0·08 g.) in methanol (1 c.c.). The red crystalline condensation product (0·18 g.) began to separate almost immediately. *Dimethin*[2-(1 : 3 : 3-*trimethylindoline*][4-(2-*carbethoxythio*-5-*thiazolone*)] crystallised from methanol in red needles, m. p. 178° (decomp.) (Found: C, 58·4; H, 5·2; N, 7·1. $C_{19}H_{20}O_3N_2S_3$ requires C, 58·7; H, 5·2; N, 7·2%).

We thank the Therapeutic Corporation of Great Britain, Ltd., the Medical Research Council, and the Rockefeller Foundation for assistance, and Sir Ian Heilbron, D.S.O., F.R.S., for his interest.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
LONDON, S.W.7.

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307. *Studies in the Azole Series. Part XVI. Synthesis of a New Analogue of Penicillamine.*

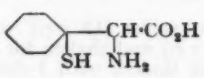
By J. D. BILLIMORIA, A. H. COOK, and SIR IAN HEILBRON.

2-Thio-5-thiazolidone (II) has been converted into an analogue (I) of penicillamine *via* the compounds (III) and (V). The acid (I), when condensed with 2-benzyl-4-ethoxymethyleneoxazolone, affords small antibacterial activities which are due, it is believed, to a product skeletally similar to the natural penicillins.

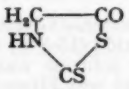
PART V of this series (Cook, Harris, and Heilbron, *J.*, 1948, 1060) described the synthesis of β-phenylcysteine which was obviously comparable with ββ-dimethylcysteine (penicillamine). However, β-phenylcysteine failed to give any significant antibiotic activity on attempted condensation with 2-benzyl-4-hydroxymethyleneoxazolone. The present paper describes the preparation and properties of an α-*amino*-β-*mercapto*-*acid* (I) more closely related to penicillamine.

2-Thio-5-thiazolidone (II) (Cook, Heilbron, and Levy, *J.*, 1948, 201) has been found to undergo facile condensation with aldehydes or ketones; thus, *cyclo*hexanone in an acid or basic medium

afforded 2-*thio*-4-cyclo*hexylidene*-5-*thiazolidone* (III) together with a by-product which the analytical evidence showed was derived by the condensation of two molecules of *cyclo*hexanone



(I.)



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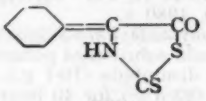
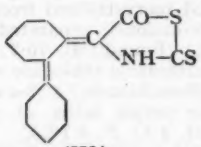
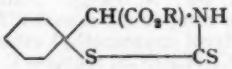


(III.)

with one of the thiazolidone and which must be the *compound* (IV). When (III) was treated with an excess of alcoholic alkali it was converted by the formal addition of one molecule of water into an *acid* which, on the basis of similar transformations associated with syntheses of β-phenylcysteine and penicillamine (Cook, Harris, and Heilbron, *loc. cit.*), can only be (V; R = H).



(IV.)



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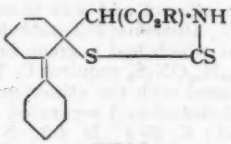
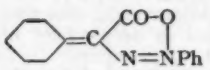


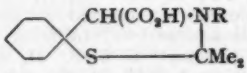
(VI.)

When only 1 mol. of sodium ethoxide was used in this transformation the product was the corresponding *ester* (V; R = Et) which was hydrolysed to the parent acid by means of alkali. The bis-condensation product (IV) underwent similar changes to give the *ester* and *acid* (VI; R = Et and H, respectively); of these the ester could be obtained directly from the mother-liquors obtained in the preparation of (III) by treatment with ethanol. The 2-thiothiazolidine (V; R = H or Et) was readily cleaved on heating with mineral acid to give the desired α-amino-β-mercapto-acid (I).

A synthesis of the latter acid *via* 4-cyclo*hexylidene*-2-*phenyl*-5-*oxazolone* (VII) was abandoned owing to the poor yield of condensation product obtained during azlactonisation of hippuric acid in the presence of *cyclo*hexanone.

(VII.)





(VIII.)

Like other acids of its class, (I) gave an intense blue-violet coloration with ferric chloride, a sparingly soluble mercury derivative, and on condensing with acetone was converted into a *thiazolidine* (VIII; R = H) which afforded a characteristic N-*formyl* derivative (VIII; R = CHO).

The aminomercapto-acid (I) was condensed with 2-benzyl-4-ethoxymethylene-5-oxazolone under conditions similar to those which afforded penicillin from penicillamine (see, *e.g.*, du Vigneaud, Carpenter, Holby, Livermore, and Rachel, *Science*, 1946, **104**, 431). Small antibacterial activities equivalent to *ca.* 0·5 penicillin unit/mg. of aminomercapto-acid were constantly obtained. The active material could be extracted by organic solvents and it seems probable that the activity is due to a product with a constitution similar to that of the natural penicillins. Attempts are consequently being made to examine the behaviour in this respect of the optically active forms of the acid (I).

Experimental.

2-*Thio*-4-cyclo*hexylidene*-5-*thiazolidone* (III).—(*a*) 2-Thio-5-thiazolidone (Cook, Heilbron, and Levy, *loc. cit.*) (25 g.) was dissolved in warm *cyclo*hexanone (75 c.c.), the solution cooled to room temperature, and ethanol (0·2 c.c.) added. Dry hydrogen chloride was passed through the solution throughout. An exothermic reaction set in and after 2 hours the solution was cooled to 0°. The crystals were filtered off from the viscous solution, washed with ethanol, and recrystallised from acetic acid, giving the *thiazolidone* (III) (10 g.) as pale yellow needles, m. p. 225° (decomp.) (Found: C, 50·4; H, 5·15; N, 6·25. $C_9H_{11}ONS_2$ requires C, 50·7; H, 5·15; N, 6·55%).

(*b*) 2-Thio-5-thiazolidone (2 g.) was dissolved in dry *cyclo*hexanone (7 c.c.) at 140°, morpholine (1 drop) added, and the solution kept at room temperature. Morpholine (1 drop) was added to the solution every 24 hours during 5 days. The solution was diluted with acetic acid, cooled to 5°, and the crystalline condensation product filtered off and recrystallised from acetic acid, forming pale yellow needles (yield 1·8 g.), m. p. 225° (decomp.) undepressed by admixture with the above condensation product.

(*c*) 2-Thio-5-thiazolidone (10 g.) was dissolved in boiling acetic acid (100 c.c.), and *cyclo*hexanone diethylketal (12 c.c.) added followed by piperidine (2 drops). The solution was kept for 24 hours and the crystalline condensation product (11·5 g.) was filtered off; it had m. p. 225° (decomp.) and was identified with the preceding preparations.

2-*Thio*-4-*carbethoxy*-(2′-cyclo*hexylidene*-5 : 5-*pentamethylene*)*thiazolidine* (VI; R = Et).—The viscous filtrate from the above condensation (*a*) was diluted with ethanol, excess of *cyclo*hexanone, ethanol, and hydrogen chloride removed by heating in a vacuum, and the residual oil poured into a mixture of much light petroleum and water. After standing at 0° for 2 days a crystalline mass separated and was filtered off. The product crystallised from acetic acid as pale yellow needles, m. p. 125—126° (yield 8 g.). Analysis indicated that this was the rearranged *ester* of the 2-thiothiazolidine described below (Found: C, 60·1; H, 7·4; S, 18·5. $C_{17}H_{25}O_2NS_2$ requires C, 60·2; H, 7·4; S, 18·9%).

2-*Thio*-4-(2′-cyclo*hexylidene*)cyclo*hexylidene*-5-*thiazolidone* (IV).—2-Thio-5-thiazolidone (5 g.) was dissolved in warm *cyclo*hexanone, and dry hydrogen chloride passed through the solution for 6 hours. The solution was kept overnight, and small amounts of crystalline material (2-thio-4-*cyclo*hexylidene-5-thiazolidone) were filtered off. The mother-liquor was poured into a mixture of much light petroleum and water, and the solution kept for 2 days at 0°. The solid was filtered off and crystallised from ethanol–water. Recrystallisation from ethanol–water yielded pale yellow needles, m. p. 203—205° (decomp.), of the hydrated bis-condensation *product* (IV) (2 g.) (Found: C, 57·6; H, 6·75; N, 4·2. $C_{15}H_{19}ONS_2,H_2O$ requires C, 57·85; H, 6·95; N, 4·5%).

2-*Thio*-4-*carboxy*-(2′-*cyclohexylidene*-5 : 5-*pentamethylene*)*thiazolidine* (VI; R = H).—The above bis-condensation product (5 g.) was suspended in ethanol (20 c.c.), and to this was added an excess of alcoholic sodium ethoxide solution (from 2 g. of sodium). The solution was heated under reflux for 5 minutes, kept at room temp. for 1 hour, and poured on crushed ice (50 g.) and hydrochloric acid (50 g.). The precipitated *acid* (VI; R = H) was filtered off, dissolved in aqueous sodium hydrogen carbonate, and the solution acidified. The acid so obtained crystallised from acetic acid in yellow prisms (5 g.), m. p. 203—205° (decomp.) (Found: C, 57·7; H, 7·0; S, 20·3. $C_{15}H_{21}ONS_2$ requires C, 57·9; H, 6·75; S, 20·6%). Its ester (VI; R = Et) (1 g.) (described above) was heated under reflux for 15 minutes with aqueous alcoholic 5% sodium hydroxide (20 c.c.). After cooling, the solution was acidified, and the precipitated acid purified by solution in aqueous sodium hydrogen carbonate and reprecipitation with acid. The acid crystallised from acetic acid in yellow prisms, m. p. 203—205°, identical with the above preparation.

2-*Thio*-4-*carbethoxy*-5 : 5-*pentamethylenethiazolidine* (V; R = Et).—The thiazolidone (III) (above) (25 g.) was suspended in dry ethanol (125 c.c.) to which was added a saturated solution of alcoholic sodium ethoxide (from 4 g. of sodium). The thiazolidone immediately dissolved. The solution was heated under reflux for 3—4 minutes and poured on crushed ice (100 g.) and hydrochloric acid (100 g.), a gum separating. The solution was diluted with water, (1500 c.c.) and kept at 0° for 12 hours, whereupon the gum solidified. The solid was filtered off and a small portion sublimed in a high vacuum, a pale yellow crystalline sublimate, m. p. 136—138°, being obtained. The remaining solid was dissolved in acetone, and the solution treated with charcoal, and seeded with the sublimate; the *ester* crystallised, and recrystallisation from aqueous acetone gave almost colourless needles (27 g.) (Found: C, 50·75; H, 6·6; N, 5·85. $C_{11}H_{17}O_2NS_2$ requires C, 50·95; H, 6·6; N, 5·45%).

2-*Thio*-4-*carboxy*-5 : 5-*pentamethylenethiazolidine* (V; R = H).—2-Thio-4-*cyclo*hexylidenethiazolidone (2 g.) was heated under reflux for 4 minutes with ethanol (10 c.c.) and an excess of sodium ethoxide (from 2 g. of sodium). The product was poured on crushed ice and hydrochloric acid. The precipitated *acid* was purified by dissolving it in sodium hydrogen carbonate and reprecipitating it with acid, and crystallised from acetic acid as colourless needles, m. p. 203—205° (decomp.) (Found: C, 46·45; H, 5·65; N, 5·7. $C_9H_{13}O_2NS_2$ requires C, 46·75; H, 5·8; N, 6·0%). The same acid was obtained by heating the above ester (1 g.) under reflux with 5% aqueous alcoholic sodium hydroxide solution (20 c.c.) and purifying the crude acid in the usual manner.

α-Amino-β-mercapto-ββ-pentamethylenepropionic Acid Hydrochloride.—(*a*) The foregoing thiazolidine ester (V; R = Et) (2 g.) was heated with hydrochloric acid (20 c.c.) under pressure at 110° for 36 hours. The solution was evaporated to dryness in a vacuum under nitrogen. The gummy residue was dissolved in water, the solution filtered, and saturated aqueous mercuric chloride added until no further precipitation occurred. The white curdy precipitate of the mercury complex was suspended in ethyl acetate (20 c.c.), previously moistened with 2N-hydrochloric acid, and hydrogen sulphide passed through the solution. The solution was filtered from mercuric sulphide, and the filtrate, after expulsion of hydrogen sulphide, was saturated with hydrogen chloride and evaporated to dryness in a vacuum. The residual white solid crystallised from a small volume of warm acetic acid after addition of light petroleum as needles, m. p. 222—223° (decomp.).

(*b*) The thiothiazolidine ester (20 g.) was heated with concentrated hydrochloric acid (80 c.c.) (sealed vessel) at 140° for 24 hours, and the solution kept at room temperature for 24 hours; the amino-mercapto-acid *hydrochloride* crystallised out in thick needles (13 g.), m. p. 223—224° (frothing, decomp.). A portion was recrystallised from cold methanol by the addition of ether, and the m. p. was unchanged (Found: C, 42·5; H, 7·1; N, 6·1. $C_8H_{16}O_2NSCl$ requires C, 42·6; H, 7·1; N, 6·2%). The acid hydrochloride when neutralised with aqueous sodium hydrogen carbonate and treated with neutral ferric chloride solution gave an intense violet coloration.

4-*Carboxy*-5 : 5-*pentamethylene*-2 : 2-*dimethylthiazolidine Hydrochloride* (VIII; R = H).—The above acid (5 g.) was suspended in dry acetone (30 c.c.), acetone saturated with hydrogen chloride (2 drops) was added, and the mixture heated under reflux under nitrogen for 1 hour. The reaction proceeded throughout in suspension. After cooling to 0° for 1 hour the *thiazolidine hydrochloride* was filtered off as colourless micro-needles (6 g.), m. p. 215—217° (frothing). It recrystallised from acetic acid–ether in colourless needles with the same m. p. (Found: C, 50·0; H, 7·6; N, 5·4; Cl, 13·4; S, 11·6. $C_{11}H_{20}O_2NSCl$ requires C, 49·70; H, 7·6; N, 5·3; Cl, 13·5; S, 12·1%).

Formyl derivative. The above thiazolidine hydrochloride (9 g.) was added to pyridine (9 c.c.). The mixture was warmed for a few minutes and the excess of pyridine removed in a high vacuum at 0°. Without removal of the pyridine hydrochloride, the residual crust was dissolved in 98—100% formic acid (100 c.c.) and, with mechanical stirring and cooling in cold water, acetic anhydride (35 c.c.) was run in dropwise during 5 hours. Water (35 c.c.) was then run in dropwise with stirring, and the solution kept at room temperature for 12 hours. The *N-formyl* derivative separated in stout needles. The

crystals were filtered off, and the mother-liquor evaporated almost to dryness, a second crop being obtained; the total yield was 10·2 g. Recrystallisation from aqueous acetic acid gave silky needles, m. p. 201—202° (Found: C, 56·1; H, 7·4. $C_{12}H_{19}O_2NS$ requires C, 56·0; H, 7·45%).

The above *N*-formylthiazolidine (0·5 g.) was heated with 2N-hydrochloric acid (10 c.c.) under nitrogen for 1 hour. The solution was evaporated in a vacuum and the free amino-mercapto-acid hydrochloride, m. p. 223—224° (frothing), was obtained, undepressed when melted in admixture with the previously prepared sample.

Penicillin Condensation.—(*a*) The amino-mercapto-acid (11 mg.), pyridine (2·5 c.c.), and an excess of 2-benzyl-4-ethoxymethylene-5-oxazolone (22 mg.) were warmed on the water-bath for 0·5 hour. Dry pyridine hydrochloride (0·6 mg.) was then added, and the solution heated in an oil-bath for 10 minutes (external temp. 120°). The reaction mixture was rapidly chilled, and the pyridine removed in a high vacuum at 0°. The residue (*ca.* 0·2—0·5 c.c.) was buffered with 5% phosphate buffer (pH 7) (5 c.c.), and the total volume made up with buffer to 11 c.c. (Solution A). Three blank solutions, B, C, and D were prepared. (B) The amino-mercapto-acid (3 mg.) treated under the condensation conditions without the oxazolone (final volume, 3 c.c.). (C) The oxazolone (6 mg.) treated under the condensation conditions (final volume, 3 c.c.). (D) Pyridine (2·5 c.c.) and pyridine hydrochloride (0·6 mg.) treated under the condensation conditions (final volume, 11 c.c.).

Plate tests (*Staph. aureus*) indicated an inhibition zone of 6 mm. diam. corresponding to *ca.* 0·5 penicillin unit of activity per c.c. for solution A. Solutions B, C, and D showed no inhibition.

(*b*) The above condensation was repeated with the amino-mercapto-acid (200 mg.), pyridine (12 c.c.), and the oxazolone (210 mg.). The total buffer solution was made up to 30 c.c. Total activity 90 units (3 units per c.c.). The solution was cooled and acidified with 1N-phosphoric acid to pH 2·8 and rapidly extracted into ethyl acetate (50 c.c.). The ethyl acetate layer was separated and washed with a small quantity of cold water. The activity was then transferred by shaking with phosphate buffer solution (pH 7·2) (20 c.c.). The buffer solution gave an activity of 40 penicillin units.

4-cyclo*Hexylidene-2-phenyl-5-oxazolone* (VII).—Hippuric acid (40 g.) was powdered and mixed with finely powdered, freshly fused, sodium acetate (50 g.). The mixture was suspended in acetic anhydride (120 c.c.) and *cyclo*hexanone (22 c.c.) and heated for 1 hour on the steam-bath. The product was poured into crushed ice (200 g.) and light petroleum (400 c.c.). A gum separated which solidified on standing for some hours at 0°. The *oxazolone* (VII), filtered off and crystallised from aqueous ethanol, had m. p. 135—137°. A recrystallisation from aqueous ethanol yielded salmon-pink needles (5 g.), m. p. 135—137° (Found: C, 74·5; H, 6·2; N, 5·95. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·2; N, 5·8%).

We thank the Therapeutic Research Corporation of Great Britain, Ltd., for assistance.

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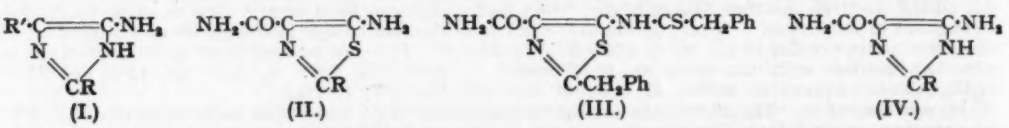
308. *Studies in the Azole Series. Part XVII. The Preparation and Cyclisation Reactions of Aminocyanoacetamide.*

By A. H. COOK, SIR IAN HEILBRON, and E. SMITH.

Aminocyanoacetamide has been prepared and cyclised to a variety of 5-aminothiazole-4-carboxyamides and 5-aminoglyoxaline-4-carboxyamides.

RECENT parts of this series (Parts XI—XIV, this vol., pp. 1061 *et seq.*) described the use of ethyl aminocyanoacetate in the synthesis of 5-amino-4-carbethoxy-thiazoles and -glyoxalines (I; R′ = CO_2Et) and their further conversion into thiazolopyrimidines and purines, respectively. The latter syntheses comprise variants on a possible more useful approach to purines *via* the unknown 5-amino-4-cyanoglyoxalines (I; R′ = CN). This approach, which is at present under investigation, seeks to complete the pyrimidine ring of the purine system by linking the amino- and the cyano-substituent in (I; R′ = CN) with the facility with which these groups of an α-amino-nitrile may be locked into a glyoxaline ring, as was earlier described. Two routes for the preparation of the requisite compounds (I; R′ = CN) at once claim attention; they are (*a*) the preparation and dehydration of appropriate amides (I; R′ = $CO{\cdot}NH_2$), and (*b*) the preparation and cyclisation of the hitherto undescribed aminomalonitrile. The present communication describes experiments directed towards (*a*), together with some related syntheses.



When ethyl aminocyanoacetate was kept with aqueous ammonia at 0°, *aminocyanoacetamide* rapidly separated. Like other α-amino-nitriles studied in this series, the new compound reacted smoothly with carbon disulphide to give a pseudo-acidic compound which must be

5-amino-2-mercaptothiazole-4-carboxyamide (II; R = SH), for on methylation in alkaline solution it was converted into the alkali-insoluble *5-amino-2-methylthiothiazole-4-carboxyamide* (II; R = SMe), which could be diazotised and was characterised as the corresponding *5-formamido-* and *5-benzamido*-compound as well as a *diacetyl* compound of unelucidated constitution.

Again, treatment of aminocyanoacetamide with an equimolecular quantity of ethanolic phenyldithioacetic acid gave a new diazotisable base (cf. Cook, Heilbron, and Levy, *J.*, 1947, 1594), *5-amino-2-benzylthiazole-4-carboxyamide* (II; R = CH_2Ph); an excess of the dithio-acid with either aminocyanoacetamide or (II; R = CH_2Ph) gave a compound which could not be diazotised and must be *5-phenylthioacetamido-2-benzylthiazole-4-carboxyamide* (III). A similar reaction between aminocyanoacetamide and sodium dithioformate in cold aqueous solution led to yet another diazotisable base formulated as *5-aminothiazole-4-carboxyamide* (II; R = H).

α-Amino-nitriles have been found to react with *iso*thiocyanates (Cook, Downer, and Heilbron, *J.*, 1948, 1262) to yield in most cases substituted 2 : 4-diaminothiazoles. Aminocyanoacetamide behaved similarly in this respect, reacting with methyl *iso*thiocyanate to give *5-amino-2-methylaminothiazole-4-carboxyamide* (II; R = NHMe). Like others of its class, the last compound was converted into a pseudo-acidic isomeride on treatment with alkali; the isomeride was identical with 5-amino-2-mercapto-1-methylglyoxaline-4-carboxyamide, prepared earlier (*idem, ibid.*) by the interaction of the corresponding ethyl glyoxalinecarboxylate and ammonia. Aminocyanoacetamide reacted similarly with carbethoxy *iso*thiocyanate and also with benzoyl *iso*thiocyanate to give diazotisable bases which are analogously formulated as *5-amino-2-carbethoxyamino-* and *5-amino-2-benzamido-thiazole-4-carboxyamide* respectively.

Finally, it was earlier shown (Cook, Davis, Heilbron, and Thomas, *loc. cit.*) that α-amino-nitriles reacted with thioiminoethers or with formamidine to give 5-aminoglyoxalines. In parallel fashion aminocyanoacetamide and formamidine afforded 5-aminoglyoxaline-4-carboxyamide (IV; R = H), and from thioacetiminobenzyl ether there was obtained *5-amino-2-methylglyoxaline-4-carboxyamide* (IV; R = Me).

The glyoxaline (IV; R = H) and the corresponding 3-methyl compound (Sarasin and Wegmann, *Helv. Chim. Acta*, 1924, **7**, 713) have hitherto been the only known 5-aminoglyoxaline-4-carboxyamides. The former is of particular interest as it appears to be a natural precursor of purines in bacterial synthesis, and accumulates in cultures of *E. coli.* in presence of sublethal quantities of sulphanilamide (Shrive, Ackermann, Gordon, Getzendaner, and Eakin, *J. Amer. Chem. Soc.*, 1947, **69**, 725). The present synthesis makes this compound (as well as its analogues) easily accessible, and its natural occurrence lends significance to the conversion of both it and its analogues into purines to be described in a subsequent communication.

Experimental.

Preparation of Aminocyanoacetamide.—A solution of ethyl aminocyanoacetate (25 g.) in ether (35 c.c.) was cooled to 0° and stirred with ice-cold aqueous ammonia (35 c.c., *d* 0·88). Crystals began to separate after 15 minutes: the solution was stirred occasionally for a further 15 minutes and then filtered. The *aminocyanoacetamide* (11 g.), crystallised from ethyl acetate, separated in glistening white rhombohedral leaflets, m. p. 121° (Found: C, 36·8; H, 5·4; N, 42·5. $C_3H_3ON_3$ requires C, 36·4; H, 5·2; N, 42·4%).

Reaction of Aminocyanoacetamide with Carbon Disulphide.—Aminocyanoacetamide (2·0 g.) was covered with methanol (20 c.c.), and the solution was heated under reflux with excess of carbon disulphide (5 c.c.) for 30 minutes, a yellow solid (2·1 g.) separating from the hot solution as micro-laths. It was purified by dissolving it in 1N-aqueous sodium carbonate and precipitating it with 1N-hydrochloric acid. It was formulated as *5-amino-2-mercaptothiazole-4-carboxyamide* (II; R = SH), m. p. 239° (decomp.) (Found: C, 27·8; H, 2·8; N, 23·8. $C_4H_3ON_3S_2$ requires C, 27·4; H, 2·9; N, 24·0%).

The thiazole (2·0 g.) was methylated by dissolving it in 1N-aqueous sodium hydroxide (5 c.c.), and shaking the solution with an excess (3 c.c.) of methyl sulphate. After 2—3 minutes the solution set to a solid mass and the product, *5-amino-2-methylthiothiazole-4-carboxyamide* (II; R = SMe), m. p. 148°, was collected (2·0 g.), washed with water (20 c.c.) followed by ether (5 c.c.), and recrystallised from ethanol, forming long, thin, slightly tapered rods, m. p. 148° (Found: C, 31·9; H, 3·9; N, 21·9. $C_5H_7ON_3S_2$ requires C, 31·7; H, 3·7; N, 22·2%). (i) This amide (0·5 g.) was heated under reflux for 4 hours with acetic anhydride (100 c.c.). The excess of reagent was removed under reduced pressure, and the residue was extracted with hot ethanol (8 c.c.). On cooling, crystals (0·6 g.) of the *diacetyl* derivative separated, and recrystallised from ethanol in laths, m. p. 192° (Found: C, 39·9; H, 4·0; N, 15·7. $C_9H_{11}O_3N_3S_2$ requires C, 39·6; H, 4·05; N, 15·4%). (ii) 5-Amino-2-methylthiothiazole-4-carboxyamide (1·0 g.) was dissolved in ether (125 c.c.), and the solution treated with an excess of benzoyl chloride (2·0 g.) and shaken for 2 hours with 1N-aqueous sodium hydrogen carbonate (100 c.c.). The *benzoyl* derivative (1·5 g.) was filtered off, dried, and recrystallised from glacial acetic acid, forming laths, m. p. 202° (Found: C, 48·9; H, 3·9; N, 14·3. $C_{12}H_{11}O_3N_3S_2$ requires C, 49·1; H, 3·8; N, 14·3%). (iii) The original amide (0·5 g.) was heated under reflux for 2 hours with formic acid (12 c.c.) and acetic anhydride (12 c.c.), and the solvents removed under reduced pressure. The residue was

dissolved in the minimum quantity of hot acetic acid (5 c.c.), and methanol (15 c.c.) added. The crystals (0·4 g.) of the N-*formyl* derivative recrystallised from glacial acetic acid in laths, m. p. 219·5—220° (Found: N, 19·2. $C_6H_7O_2N_3S_2$ requires N, 19·3%).

Reaction of Aminocyanoacetamide with Phenyldithioacetic Acid.—(i) Aminocyanoacetamide (1·0 g.) was covered with ethanol (35 c.c.), and the solution heated under reflux for 1½ hours with an excess (3·5 g.) of phenyldithioacetic acid in ether (10 c.c.) and then diluted with crushed ice (120 g.). The milky solution was kept at 0° (1 hour) and then filtered. The product, *5-phenylthioacetamido-2-benzylthiazole-4-carboxyamide* (III) (1·9 g.), was washed with ether (20 c.c.), and recrystallised from benzene in thin, white, tapered rods, m. p. 163·5° (Found: C, 62·0; H, 5·1; N, 11·4. $C_{19}H_{17}ON_3S_2$ requires C, 62·1; H, 4·7; N, 11·4%).

(ii) The preceding experiment was repeated with phenyldithioacetic acid (1·7 g.) in ether (10 c.c.) and aminocyanoacetamide (1·0 g.). The product (0·85 g.) was washed with ether, and *5-amino-2-benzylthiazole-4-carboxyamide* (II; R = CH_2Ph) was recrystallised from toluene, forming clusters of bifurcated plume-shaped blades, m. p. 144° (Found: C, 56·6; H, 4·5. $C_{11}H_{11}ON_3S$ requires C, 56·8; H, 4·8%).

Reaction of Aminocyanoacetamide with Sodium Dithioformate.—Aminocyanoacetamide (0·5 g.) and sodium dithioformate (0·8 g.) were dissolved in water (4 c.c.). The solution was left at room temperature for 12 hours and then at 0° for 24 hours. The crystals which appeared (0·1 g.) were filtered off and the filtrate was extracted three times with ether (50 c.c.). The extract was concentrated, leaving further yellow crystals. The combined product, *5-aminothiazole-4-carboxyamide* (II; R = H) (0·3 g.), crystallised from toluene in laths, m. p. 140—141° (Found: N, 29·2. $C_4H_5ON_3S$ requires N, 29·3%).

Reaction of Aminocyanoacetamide with Methyl iso*Thiocyanate.*—Aminocyanoacetamide (1·0 g.) was heated under reflux with ethyl acetate (80 c.c.) and methyl *iso*thiocyanate (0·7 g.) for 0·5 hour. The solvent was removed under reduced pressure, and the residue crystallised from methanol. *5-Amino-2-methylaminothiazole-4-carboxyamide* (II; R = NHMe) (1·1 g.) separated, m. p. 178° (Found: C, 35·2; H, 4·9; N, 32·8. $C_5H_8ON_4S$ requires C, 34·9; H, 4·7; N, 32·5%).

The preceding compound (1·2 g.) was heated under reflux for 1 hour with 1N-aqueous sodium carbonate (10 c.c.). The solution was then made just acid to litmus with 1N-hydrochloric acid. 5-Amino-2-mercapto-1-methylglyoxaline-4-carboxyamide (0·9 g.) separated, and recrystallised from methanol in laths, m. p. 245°; it gave no depression on mixed m. p. determination with authentic material.

Reaction of Aminocyanoacetamide with Carbethoxy iso*Thiocyanate.*—Aminocyanoacetamide (1·0 g.) was heated under reflux with acetone (40 c.c.) and the *iso*thiocyanate (1·3 g.) for 15 minutes. The solution was cooled and diluted with crushed ice (150 g.). The product (1·6 g.) was crystallised from ethanol, *5-amino-2-carbethoxyaminothiazole-4-carboxyamide* (II; R = NH·CO_2Et) separating in laths which gradually blackened up to 400° without melting (Found: C, 36·5; H, 4·3; N, 23·9. $C_7H_{10}O_3N_4S$ requires C, 36·5; H, 4·4; N, 24·3%).

Reaction of Aminocyanoacetamide with Benzoyl iso*Thiocyanate.*—Aminocyanoacetamide (1·0 g.) and benzoyl *iso*thiocyanate (1·6 g.) were heated under reflux with acetone (30 c.c.) for 15 minutes, a dense yellow precipitate of *5-amino-2-benzamidothiazole-4-carboxyamide* (II; R = NH·COPh) being formed. The solution was cooled and the product (1·8 g.) was collected and washed with acetone and with water. It was crystallised from aqueous pyridine in clusters of fine white needles, m. p. 285° (Found: C, 50·5; H, 4·2; N, 21·4. $C_{11}H_{10}O_2N_4S$ requires C, 50·4; H, 3·9; N, 21·4%).

Reaction of Aminocyanoacetamide with Thioacetiminobenzyl Ether Hydrochloride.—Aminocyanoacetamide (0·5 g.) and the thioiminoether hydrochloride (1·0 g.) were dissolved in dry pyridine (5 c.c.). The solution was kept at 80° (10 minutes), then set aside overnight at 0°. The product, *5-amino-2-methylglyoxaline-4-carboxyamide hydrochloride*, m. p. 238—240°, was collected, and crystallised from methanol–ether in colourless prisms (Found: C, 33·6; H, 5·6; N, 31·5. $C_5H_9ON_4Cl$ requires C, 34·0; H, 5·1; N, 31·7%).

Reaction of Aminocyanoacetamide with Formamidine Hydrochloride.—Aminocyanoacetamide (5·0 g.) and formamidine hydrochloride (4·0 g.) were heated under reflux in methanol (20 c.c.) for 1 hour. Attempts to isolate the free base led to a brown diazotisable oil, and the solution was treated with a saturated solution of picric acid (10 g.) in methanol. On cooling, a picrate (5·4 g.), m. p. 210—214°, separated, and was recrystallised repeatedly from ethanol to give yellow needles, m. p. 236° (decomp.). The picrate was formulated as that of 4-aminoglyoxaline-5-carboxyamide (IV; R = H), for which Windaus and Langenbeck (*Ber.*, 1923, **56**, 683) report m. p. 240° (decomp.) (Found: C, 33·4; H, 3·4; N, 26·3. Calc. for $C_4H_6ON_4,C_6H_3O_7N_3$: C, 33·8; H, 3·1; N, 27·6%). The low value for nitrogen found for this compound has been commented upon elsewhere (Stetten and Fox, *J. Biol. Chem.*, 1945, **161**, 333).

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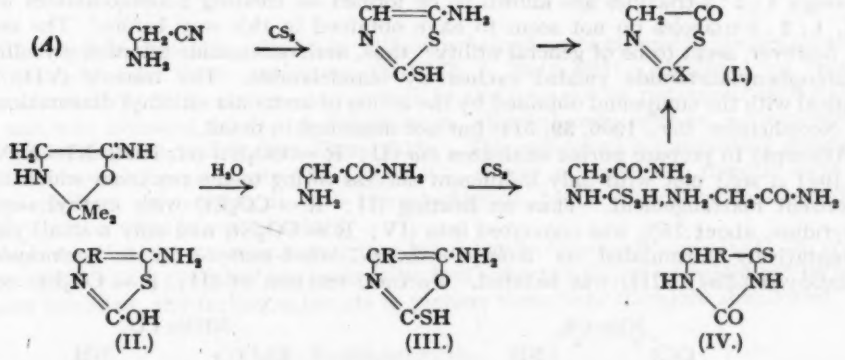
309. *Studies in the Azole Series. Part XVIII. The Interaction of α-Amino-nitriles and Carbon Oxysulphide.*

By A. H. Cook, Sir Ian Heilbron, and G. D. Hunter.

Interaction of carbon oxysulphide and α-amino-nitriles affords 5-amino-2-hydroxythiazoles (II). The latter are similar to but less stable than structurally comparable 2-mercaptothiazoles, readily undergoing cleavage or rearrangement to give 4-thiohydantoins (IV).

2-Mercapto-5-thiazolone (I; X = SH, or a tautomeride thereof) (Part III of this series, *J.*, 1948, 201) has proved useful in the synthesis of amino-acids, aminomercapto-acids, and polypeptides (Cook, Heilbron, *et al.*, *Nature*, in the press). In certain cases, however, the readiness with which the necessary ring-fissions and rearrangements take place leaves some room for improvement. It was accordingly thought that in these respects the so far unknown 2-hydroxy-5-thiazolone (2 : 5-diketothiazolidine) (I; X = OH, or its tautomeride) and its 4-substituted derivatives might exhibit the required activity, intermediate between that of *N*-carboxyglycine anhydride and of (I; X = SH). The latter compound has been prepared by two related routes (*A*) and (*B*); the present paper gives the results of experiments designed to simulate these methods with either aminoacetonitrile or other α-amino-nitriles, the carbon disulphide being replaced by carbon oxysulphide.



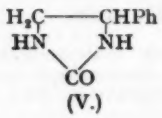
As early experiments with aminoacetonitrile and carbon oxysulphide usually led to tarry products, attention was first concentrated on the behaviour of more stable α-amino-nitriles.

Reaction between α-aminobenzyl cyanide and carbon oxysulphide in ethanolic solution led to a compound for which, on analytical evidence, structures (II and III; R = Ph) came into consideration. A more remote possibility represented by (IV; R = Ph) was eliminated by direct comparison of the new compound with 4-thio-5-phenylhydantoin (Johnson and Chernoff, *J. Amer. Chem. Soc.*, 1912, **34**, 1208). The latter substance was characterised as its *monomethyl* and its *acetyl* derivative. An attempt to desulphurise the thiohydantoin with alkaline hydrogen peroxide led to the formation of the corresponding *disulphide*. The compound (II or III; R = Ph) was indeed converted into (IV) when it was treated either with cold aqueous sodium hydroxide or with Raney nickel. Clearly, (II) is more acceptable than (III; R = Ph) as representing the immediate precursor of the 4-thio-5-phenylhydantoin, and the compound under consideration is thus regarded as *5-amino-2-hydroxy-4-phenylthiazole* (II; R = Ph) (or a tautomeric form). A second product from the treatment of (II; R = Ph) or of (IV; R = Ph) with Raney nickel was 4-phenyl-2-iminazolidone (V); the latter had previously been obtained by a tedious method (Karewskaja, *J. pr. Chem.*, 1932, **132**, 335) but there was no doubt of the identity of the material obtained during the present work as it was hydrolysed to α-phenylethylenediamine which was characterised as its known acetyl derivative.

5-Amino-2-hydroxy-4-phenylthiazole yielded a *hydrochloride* and a *diacetyl* derivative. It condensed with benzaldehyde to give a *benzylidene* derivative, and, also like other 5-aminothiazoles (cf. Part II, *J.*, 1947, 1598), it developed a bright red colour on warming with ethanolic glyoxal.

Reaction between ethyl aminocyanoacetate and ethereal carbon oxysulphide resulted in a compound which, by analogy with one of those described above and from its behaviour, must be *5-amino-2-hydroxy-4-carbethoxythiazole* (II; R = CO_2Et). It readily formed a *monoacetyl*

and a *benzylidene* derivative, and underwent rearrangement even more easily than the phenyl analogue above; thus in presence of either aqueous ammonia or sodium carbonate it was converted into *5-carbethoxy-4-thiohydantoin* (IV; R = CO_2Et), a monohydrated *sodium* salt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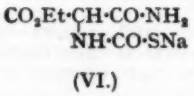
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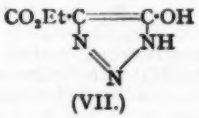
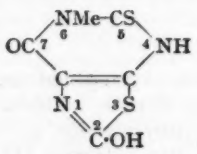

(VII.)

of the thiohydantoin being isolated as an intermediate when using sodium carbonate. Desulphurisation of either (IV; R = CO_2Et) or, with simultaneous rearrangement, of (II; R = CO_2Et) using Raney nickel afforded 2-hydroxy-4-carbethoxyglyoxaline (Hilbert, *J. Amer. Chem. Soc.*, **1932**, **54**, 3413), characterised as its *acetyl* derivative. When (II; R = CO_2Et) was subjected to the action of either aqueous sodium hydroxide or hydrochloric acid the ring was cleaved with the formation, in the latter case, of *aminocarbethoxyacetamide hydrochloride*; in the former case an unstable sodium salt, probably sodium carbethoxycarbamylmethylthiolcarbamate (VI), was obtained and shown to undergo decomposition in presence of hydrochloric acid to give the hydrochloride already mentioned. Attempts to diazotise the thiazole (II; R = CO_2Et) gave rise to what is best regarded as *5-hydroxy-4-carbethoxy-1 : 2 : 3-triazole* (VII), as it was also obtained on treating aminocarbethoxyacetamide with nitrous acid. Although 1 : 2 : 3-triazines are known to be formed on treating β-amino-amides with nitrous acid, 1 : 2 : 3-triazoles do not seem to have obtained in this way before. The method does not, however, seem to be of general utility; thus, aminoacetamide afforded glycollic acid, and α-aminophenylacetamide yielded exclusively mandelamide. The triazole (VII) is possibly identical with the compound obtained by the action of ammonia on ethyl diazomalonate (Piloty and Neresheimer, *Ber.*, **1906**, **39**, 514) but not described in detail.

Attempts to prepare purine analogues *via* (II; R = CO_2Et) (cf. Parts XI—XIV, this vol., pp. 1061 *et seq.*) met with only indifferent success owing to the readiness which the thiazole underwent rearrangement. Thus on heating (II; R = CO_2Et) with methyl *iso*thiocyanate in pyridine, about 75% was converted into (IV; R = CO_2Et) and only a small yield of what is tentatively formulated as *5-thio-2-hydroxy-7-keto-6-methyl-4 : 5 : 6 : 7-tetrahydro-(5 : 4-d)-thiazolopyrimidine* (VIII) was isolated. Further, reaction of (II; R = CO_2Et) with methyl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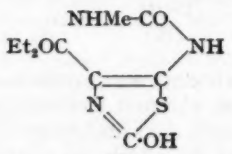
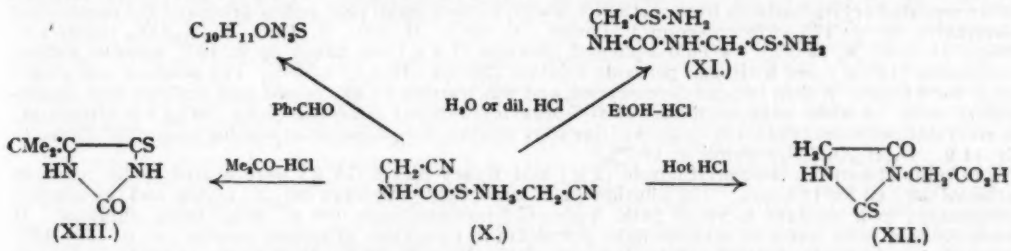

(IX.)

*iso*cyanate in pyridine afforded the expected *5-methylureido-2-hydroxy-4-carbethoxythiazole* (IX); the latter, however, could not be cyclised to a thiazolopyrimidine in presence of alkali, undergoing instead an unelucidated change.

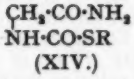
After the initial inconclusive attempts mentioned above, reaction between aminoacetonitrile and carbon oxysulphide was found to be cleanly effected in acetone solution, the immediate product being *cyanomethylammonium cyanomethylthiolcarbamate* (X), which proved to be unexpectedly reactive. For instance, on treatment with warm water, acid, or aqueous sodium carbonate, the compound liberated carbon dioxide and was converted into a new substance, $C_5H_{10}ON_4S_2$, tentatively formulated as *5-bisthiocarbamylmethylurea* (XI). Boiling (X) or (XI) with concentrated hydrochloric acid afforded 2-thiohydantoin-1-acetic acid (XII) which was in turn converted into hydantoin-1-acetic acid (Johnson and Renfrew, *J. Amer. Chem. Soc.*, **1925**, **47**, 240). Attempts to prepare 4-thiohydantoin by introduction of (X) into methanolic or ethanolic hydrogen chloride were unsuccessful, only the substance formulated as (XI) being isolated. However, when a solution of (X) in acetone was poured into aqueous mineral acid, both rearrangement and condensation occurred, *4-thio-5-isopropylidenehydantoin* (XIII) being precipitated. The structure of the last compound was indicated by its oxidation by alkaline hydrogen peroxide to *4-iso*propylidenehydantoin, identical with authentic material prepared from hydantoin. In contrast with the well-defined reaction with acetone, (X) reacted with benzaldehyde giving a crystalline *compound*, $C_{10}H_{11}ON_3S$, which was not identified.

In view of these results attention was turned to the possibility of simulating the alternative

reaction (*B*, above), using carbon oxysulphide. Interaction of aminoacetamide and carbon oxysulphide in aqueous-alcoholic solution led to *carbamylmethylammonium carbamylmethylthiol-*



carbamate (XIV; R = $NH_3 \cdot CH_2 \cdot CO \cdot NH_2$). Acidification of this salt, however, in aqueous or non-aqueous media did not effect cyclisation but led only to formation of salts of aminoacetamide with loss of carbon oxysulphide. The corresponding *potassium* salt (XIV; R = K) was prepared but it also failed to undergo cyclisation as desired. It was also of interest to find that *benzyl carbamylmethylthiolcarbamate* (XIV; R = CH_2Ph) could not be dehydrated with



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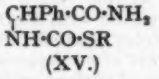

(XV.)

phosphorus tribromide or acetic anhydride, although benzyl carbamylmethyldithiocarbamate readily dehydrated under these conditions (Part III, *J.*, 1948, 201). Similarly, on treating α-aminophenylacetamide with carbon oxysulphide in the presence of potassium carbonate, a potassium salt was obtained which, on acidification, afforded only salts of α-aminophenylacetamide, potassium salts, and carbon oxysulphide. The compound is presumably potassium phenylcarbamylmethylthiocarbamate (XV; R = K); it was not analysed directly as it was very hygroscopic but, on treating it with benzyl chloride, *benzyl phenylcarbamylmethylthiolcarbamate* (XV; R = CH_2Ph) was obtained.

From these experiments it became clear that the desired compounds such as (I; X = OH) would, if accessible at all, probably have such a limited existence that they would not be useful in the manner intended, and further attempts to prepare them were therefore abandoned.

Experimental.

Reactions with α-Aminobenzyl Cyanide.—α-Aminobenzyl cyanide (21 g.) in ethanol (120 c.c.) was treated with an excess of carbon oxysulphide (30 g.), slowly bubbled through the solution during 6 hours. Bold yellow needles of *5-amino-2-hydroxy-4-phenylthiazole* (14·5 g.) were slowly formed, and a second crop (9·5 g.) was obtained from the filtrate on standing at 0° for 7 days (total yield, 24 g., 87%). The compound had m. p. 195°, immediately resolidifying and melting again at *ca.* 259° (decomp.). Although insoluble in most common organic solvents, recrystallisation was effected from pyridine–water, as pale yellow, felted needles, m. p. 255—260° (decomp.), according to the rate of heating (Found: C, 55·8; H, 4·1; S, 16·1. $C_9H_8ON_2S$ requires C, 56·2; H, 4·2; S, 16·7%). A bright red colour was obtained on warming it with ethanolic glyoxal, indicative of a 5-aminoazole structure. Acetylation by the usual procedure was very destructive, sulphur being liberated, but a 50% yield of a *diacetyl* derivative, purified by crystallisation from methanol–water, and finally aqueous acetic acid, m. p. 168—170° (decomp.), was obtained in clusters of irregular pale yellow prisms (Found: C, 56·5; H, 4·3; N, 9·8. $C_{13}H_{12}O_3N_2S$ requires C, 56·5; H, 4·3; N, 10·1%). After 5-amino-2-hydroxy-4-phenylthiazole (1·4 g.) had been shaken with saturated ethanolic hydrogen chloride (30 c.c.) for 4 hours, the almost clear yellow solution was filtered, and concentrated hydrochloric acid (60 c.c.) added. After standing at 0° for 4 days, beautiful lemon-yellow rhombohedra of the *alcoholate* of *5-amino-2-hydroxy-4-phenylthiazole hydrochloride* (1·3 g., 65%) were collected (Found: N, 10·1, 10·4. $C_9H_8ON_2S,HCl,C_2H_6O$ requires N, 10·1%). The compound rapidly lost hydrogen chloride and ethanol on standing, and in aqueous solution it immediately decomposed with deposition of starting material. On heating, it became deep orange at 120°, red-brown at 160°, dark brown at 200°, and finally melted at 260—266° (decomp.).

5-Amino-2-hydroxy-4-phenylthiazole (2·0 g.) was heated under reflux with benzaldehyde (20 c.c.) for 1 hour. The semi-solid mass was extracted with hot methanol and filtered, and the *benzylidene* derivative of 5-amino-2-hydroxy-4-phenylthiazole (0·1 g.) separated on cooling the filtrate. It recrystallised from methanol in long, yellow, hair-like needles, m. p. 271° (Found: C, 68·5; H, 4·3; N, 9·8. $C_{16}H_{12}ON_2S$ requires C, 68·6; H, 4·3; N, 10·0%).

5-Amino-2-hydroxy-4-phenylthiazole (15·0 g.) was shaken with 10% aqueous sodium hydroxide (100 c.c.) for 2 hours, a clear, pale-yellow solution being obtained. Acidification with concentrated hydrochloric acid yielded a bright yellow precipitate of 4-thio-5-phenylhydantoin (13·5 g., 90%), m. p 253—258° (decomp.), which recrystallised from 90% aqueous ethanol in small needles, m. p. 258—259° (decomp.) (Found: C, 56·6; H, 4·0. Calc. for $C_9H_8ON_2S$: C, 56·2; H, 4·2%). An alkaline solution gradually developed an orange-red colour on exposure to air. Acetylation of the thiohydantoin in the

normal way yielded a black product which on crystallisation from ethanol (charcoal) gave the *diacetyl* derivative in silvery rectangular plates, m. p. 153—154° (Found: C, 56·8; H, 4·6; N, 9·9. $C_{13}H_{13}O_3N_3S$ requires C, 56·5; H, 4·4; N, 10·1%). Methylation with diazomethane gave a yellow gum, which, after repeated crystallisations from methanol–water, formed small pale yellow prisms of the *monomethyl* derivative, m. p. 175—178° (decomp.) (Found: C, 58·1; H, 5·0; N, 13·1. $C_{10}H_{10}ON_2$ requires C, 58·2; H, 4·9; N, 13·5%). 4-Thio-5-phenylhydantoin (1·0 g.) was taken up in 10% aqueous sodium hydroxide (15 c.c.) and hydrogen peroxide solution (20-vol., 10 c.c.) added. The solution was cooled to 0° for 6 hours; it then became decolourised, and was warmed to 80°, cooled, and acidified with hydrochloric acid. A white solid precipitate of *di-5-phenylhydantoin-4 disulphide* (0·9 g., 89%) was filtered off; it recrystallised from ethanol in clusters of fine silky needles, decomposing on heating above 210° (Found: N, 14·9. $C_{18}H_{14}O_4N_4S_2$ requires N, 14·7%).

5-Amino-2-hydroxy-4-phenylthiazole (2 g.) and Raney nickel (15 g.) were heated under reflux in ethanol (30 c.c.) for 12 hours. The solution was filtered, light petroleum (30 c.c.) added, and the solution evaporated in a vacuum to small bulk, 5-phenyl-2-iminazolidone (0·6 g., 36%) being obtained. It recrystallised from water or acetone–light petroleum in colourless, glistening needles, m. p. 156—157° (Found: C, 66·6; H, 6·2; N, 17·2. Calc. for $C_9H_{10}ON_2$: C, 66·7; H, 6·2; N, 17·3%). 4-Thio-5-phenylhydantoin (1·7 g.) and Raney nickel (10 g.) in ethanol (20 c.c.) were heated under reflux for 2 hours. The solution was filtered and evaporated in a vacuum; a yellow oil was obtained, which on standing at 0° with light petroleum solidified to yield 5-phenyl-2-iminazolidone (0·3 g.). 5-Phenyl-2-iminazolidone (0·4 g.) was dissolved in concentrated hydrochloric acid (2 c.c.), the solution boiled for 5 minutes, and then evaporated to dryness. Extraction of the residue with ethanol and precipitation with ether gave α-phenylethylenediamine dihydrochloride (0·15 g., 30%), which, on recrystallisation from ethanol–ether, became yellow at 260° (cf. Karewskaja, *loc. cit.*) and finally had m. p. 313°. The hydrochloride was acylated with acetic anhydride, and the crude diacetyl derivative of α-phenylethylenediamine crystallised from benzene; m. p. 152°, in agreement with the literature value.

Reactions with Ethyl Aminocyanoacetate.—Ethyl aminocyanoacetate (27 g.) in ether (100 c.c.) was treated with an excess of carbon oxysulphide (30 g.), bubbled into the solution during 6 hours. Yellow crystals of *5-amino-2-hydroxy-4-carbethoxythiazole* (34 g., 96%) were rapidly formed, and were collected after standing at 0° for 12 hours. The compound crystallised from ethyl acetate or acetone in pale yellow tablets, m. p. 158° (Found: C, 38·6; H, 4·2; N, 14·8; S, 16·6. $C_6H_8O_3N_2S$ requires C, 38·3; H, 4·3; N, 14·9; S, 17·0%). Acetylation of the base by the usual procedure gave *5-acetamido-2-hydroxy-4-carbethoxythiazole*, which crystallised from ethanol in colourless needles, m. p. 239° (decomp.) (Found: C, 42·0; H, 4·4; N, 12·0. $C_8H_{10}O_4N_2S$ requires C, 41·7; H, 4·4; N, 12·2%).

5-Amino-2-hydroxy-4-carbethoxythiazole (2 g.) was heated for 5 minutes with benzaldehyde (20 c.c.); on cooling the solution, the *benzylidene* derivative (2·3 g., 79%) was obtained. It recrystallised from benzene in pale yellow prisms, m. p. 185° (Found: C, 57·0; H, 4·5; N, 9·8; S, 11·1. $C_{13}H_{12}O_3N_2S$ requires C, 56·5; H, 4·3; N, 10·1; S, 11·6%). A suspension of 5-amino-2-hydroxy-4-carbethoxythiazole (10 g.) in aqueous ammonia (*d* 0·880; 100 c.c.) was shaken for 12 hours, and the almost clear solution cooled to 0°. Acidification with concentrated hydrochloric acid caused precipitation of *4-thio-5-carbethoxyhydantoin* (9·9 g., 99%), which recrystallised from water, or better from ammonia–acid, in rectangular plates, m. p. 210—211° (decomp.) (Found: C, 38·4; H, 4·1; N, 14·8. $C_6H_8O_3N_2S$ requires C, 38·3; H, 4·3; N, 14·9%). An alkaline solution gave a bright cherry-red colour on exposure to air, changing overnight to a deep orange. 5-Amino-2-hydroxy-4-carbethoxythiazole (3·2 g.) was shaken with 2N-aqueous sodium carbonate (40 c.c.) for 4 hours, and the filtrate cooled to 0°; colourless massive scintillating rectangular prisms of the monohydrated *sodium* salt of 4-thio-5-carbethoxyhydantoin (1·0 g.) were filtered off and recrystallised from water–acetone, forming beautiful, long, thin, hexagonal rods which did not melt but became yellow at 170°, green at 185°, and black at 205° (Found: N, 12·2. $C_6H_7O_4N_2SNa$ requires N, 12·3%). Acidification of the salt or the filtrate gave 4-thio-5-carbethoxyhydantoin itself, identical with the product previously obtained. 4-Thio-5-carbethoxyhydantoin was characterised by acetylating it with acetic anhydride; the *diacetyl* derivative recrystallised from methanol in bundles of fine colourless needles, m. p. 160—162° (Found: C, 44·2; H, 4·4; N, 10·6. $C_{10}H_{12}O_5N_2S$ requires C, 44·1; H, 4·4; N, 10·3%). 4-Thio-5-carbethoxyhydantoin (4 g.) and Raney nickel (20 g.) in ethanol (50 c.c.) were heated under reflux for 1 hour. The hot solution was filtered, and, on cooling, colourless glistening leaflets of 2-hydroxy-4-carbethoxyiminazole (1·3 g.) appeared. A further 0·1 g. (gross yield, 59%) was obtained on adding light petroleum to the filtrate, evaporation of which gave unchanged starting material (1·1 g.). The compound crystallised from ethanol in silvery octagonal plates, m. p. 252—253° (decomp.) (Found: C, 45·9; H, 5·2; N, 17·6. Calc. for $C_6H_8O_3N_2$: C, 46·1; H, 5·1; N, 17·9%). Similar desulphurisation of 5-amino-2-hydroxy-4-carbethoxythiazole (3 g.) gave only 0·5 g. of 2-hydroxy-4-carbethoxyglyoxaline, acetylation of which with acetic anhydride gave a *monoacetyl* derivative as glistening rods, m. p. 195°, from ethanol (Found: C, 48·6; H, 5·1; N, 14·2. $C_8H_{10}O_4N_2$ requires C, 48·5; H, 5·1; N, 14·1%).

5-Amino-2-hydroxy-4-carbethoxythiazole (5 g.) was heated to 70° for 15 minutes with 4N-hydrochloric acid (40 c.c.); carbon oxysulphide was evolved, and a clear solution obtained. Evaporation to dryness in a vacuum gave an orange crystalline residue of *aminocarbethoxyacetamide hydrochloride*, which recrystallised from ethanol–ether in clusters of colourless prisms, m. p. 194—195° (decomp.) (Found: C, 32·7; H, 6·0; N, 15·1. $C_5H_{11}O_3N_2Cl$ requires C, 32·9; H, 6·0; N, 15·3%). The yield of pure hydrochloride was 3·3 g. (68%). 5-Amino-2-hydroxy-4-carbethoxythiazole (1·5 g.) dissolved readily in 10% aqueous sodium hydroxide (15 c.c.) in an atmosphere of nitrogen. Cooling to −15° for 30 minutes resulted in deposition of a colourless crystalline sodium salt (1·5 g.), m. p. 80° (decomp.), probably sodium carbethoxycarbamylmethylthiolcarbamate, which became red on exposure to air and rapidly decomposed when worked up. The experiment was repeated on the same scale, and the crystalline precipitate acidified, a clear solution being obtained. Evaporation to dryness afforded a crystalline residue from which aminocarbethoxyacetamide hydrochloride (1·0 g., 69%) was obtained by extraction with methanol and precipitation with ether.

This hydrochloride (2·0 g.) in 6N-hydrochloric acid (20 c.c.) was cooled to 0°, and a solution of sodium

nitrite (0·9 g.) in water (5 c.c.) slowly added. After cooling to 0° for 18 hours, *5-hydroxy-4-carbethoxy-1 : 2 : 3-triazole* (1·3 g., 76%) was filtered off and crystallised from benzene in colourless massive scintillating rhombohedra, m. p. 145—146° (Found : C, 38·5; H, 4·6; N, 27·6, 27·7. $C_5H_7O_3N_3$ requires C, 38·2; H, 4·5; N, 26·8%). α-Aminophenylacetamide hydrochloride (3·0 g.) in water (40 c.c.) was treated at 0° with a solution of sodium nitrite (1·3 g.) in water (6 c.c.). A vigorous effervescence occurred, and on evaporation to dryness in a vacuum, mandelamide (2·4 g.) was obtained by extraction with hot benzene. It was recrystallised from benzene–petrol, forming colourless irregular plates, m. p. 123—124° (Found : C, 63·5; H, 6·0. Calc. for $C_8H_9O_2N$: C, 63·6; H, 6·0%). Aminoacetamide hydrochloride (10·0 g.) in water (20 c.c.) was treated at 0° with sodium nitrite (12 g.) in water (20 c.c.). After standing at 0° for 24 hours, the solution was evaporated to dryness, and extraction with hot ethanol then gave glycollic acid (4·3 g.), m. p. 70—75°, identical with an authentic specimen.

5-Amino-2-hydroxy-4-carbethoxythiazole (4 g.) and methyl *iso*thiocyanate (2 g.) in pyridine (30 c.c.) were heated under reflux for 1·5 hours. The solution was cooled and poured into water (100 c.c.), a black oil (1 c.c.) separating. The supernatant liquid was poured off, and after four days at 0°, 4-thio-5-carbethoxyhydantoin (3·0 g.) was collected, identical with the material described above. The black oil was dissolved in 10% aqueous sodium hydroxide (5 c.c.) and acidified with hydrochloric acid; a brown precipitate (1 g.), decomposing on heating above 300°, was obtained. Purification by trituration with methanol and crystallisation from dilute aqueous ammonia (charcoal)–dilute acid yielded *5-thio-2-hydroxy-7-keto-6-methyl-4 : 5 : 6 : 7-tetrahydro-(5 : 4-d)-thiazolopyrimidine* in masses of colourless felted needles, decomp. >310° (Found : S, 31·0. $C_6H_5O_2N_3S_2$ requires S, 31·5%), giving a positive murexide test.

5-Amino-2-hydroxy-4-carbethoxythiazole (10 g.) and methyl *iso*cyanate (8 g.) in pyridine (40 c.c.) were heated under reflux for 1·5 hours. The pyridine and excess of methyl *iso*cyanate were evaporated off in a vacuum, and a red gum was obtained. This solidified on treatment with methanol, crystallisation of the solid from methanol giving *5-methylureido-2-hydroxy-4-carbethoxythiazole* (10·1 g., 77%) as colourless needles, m. p. 185—186° (Found : C, 39·1; H, 4·8; N, 17·2. $C_8H_{11}O_4N_3S$ requires C, 39·2; H, 4·9; N, 17·1%). The latter compound (1 g.) was taken up in warm 2N-aqueous sodium hydroxide, and the solution acidified. On cooling, a crystalline *compound* (0·2 g.) deposited, crystallising from methanol in long, thin, colourless needles, m. p. 225° (decomp.) (Found : C, 38·9; H, 5·0; N, 19·6. $C_7H_{11}O_3N_3S$ requires C, 38·7; H, 5·1; N, 19·4%).

Reactions with Aminoacetonitrile.—Acetone (400 c.c.) was stirred vigorously in a 1-l. 3-necked flask in an atmosphere of nitrogen. A solution of aminoacetonitrile (20 g.) in acetone (80 c.c.) was slowly dropped into the flask during 4 hours, a strong current of carbon oxysulphide (60 g.) being simultaneously passed through the acetone. The yellow solution was stirred for a further hour, then evaporated to 80 c.c., and light petroleum (300 c.c.) added. After cooling to 0° for 2 hours, the orange precipitate of *cyanomethylammonium cyanomethylthiolcarbamate* (28 g., 91%) was filtered off, and purified by crystallisation from acetone–light petroleum; m. p. 173° (decomp.) (Found : C, 35·1; H, 5·0; N, 33·0. $C_5H_8ON_4S$ requires C, 34·9; H, 4·7; N, 32·6%). This compound (1·5 g.) was stirred with 15% hydrochloric acid; carbon dioxide was evolved, and after standing at 0° for 1·5 hours, a light brown precipitate of *5-bisthiocarbamylmethylurea* (XI) (?) (0·6 g., 66%) was filtered off; it crystallised from water in the presence of sodium hydrosulphite (dithionite) as long, thin, glistening needles, darkening rapidly when heated above 210° (Found : C, 29·2; H, 4·9; N, 27·1; S, 30·3. $C_5H_{10}ON_4S_2$ requires C, 29·1; H, 4·9; N, 27·2; S, 31·1%). Cyanomethylammonium cyanomethylthiolcarbamate (3 g.) was shaken with 2N-aqueous sodium carbonate (50 c.c.) for 2 hours in an atmosphere of nitrogen. The urea (XI) (1 g., 55%), identical with the sample previously obtained, was collected as a light-grey solid and recrystallised as above. The same compound was also obtained (yield, 42%) by boiling cyanomethylammonium cyanomethylthiocarbamate with water in the presence of sodium hydrosulphite (dithionite).

Cyanomethylammonium cyanomethylthiocarbamate (3·0 g.) was boiled for 2 minutes with concentrated hydrochloric acid (5 c.c.). The clear solution was cooled to 0°, and after 2 hours the colourless crystalline precipitate of 2-thiohydantoin-1-acetic acid and ammonium chloride (3·5 g., 87%) was collected. After purification by crystallisation from ethanol–light petroleum, 2-thiohydantoin-1-acetic acid formed beautiful spear-shaped laths with a pearly lustre, m. p. 215—216° (decomp.) (Found : C, 34·6; H, 3·6; N, 16·2. Calc. for $C_5H_6O_2N_2S$: C, 34·5; H, 3·5; N, 16·1%). The identity of the acid was further confirmed by desulphurisation with chloroacetic acid (cf. Johnson and Renfrew, *loc. cit.*) to give hydantoin-1-acetic acid, which melted in good agreement with the literature value (190—191°) after crystallisation from ethanol. Cyanomethylammonium cyanomethylthiolcarbamate (4·5 g.) in acetone (100 c.c.) was poured into 2N-hydrochloric acid (100 c.c.); after cooling to 0° for 12 hours, the bright orange precipitate of *4-thio-5-iso**propylidenehydantoin* (2·0 g., 49%) was collected, and crystallised from ethanol–water in light orange, felted needles, m. p. 227—229° (decomp.) (Found : C, 46·6; H, 5·3; N, 17·7. $C_6H_8ON_2S$ requires C, 46·2; H, 5·1; N, 17·9%). This hydantoin (0·6 g.) was dissolved in 10% aqueous sodium hydroxide, and the reddish-brown solution treated with hydrogen peroxide solution (20-vol., 10 c.c.). The solution became hot and changed to a pale yellow. After cooling to 0° for 6 hours, warming to 60°, filtering, and acidifying with hydrochloric acid, the solution was cooled to 0° for 12 hours, *5-iso*propylidenehydantoin (0·1 g., 18%) being obtained. This compound had m. p. *ca.* 245° (decomp.), on crystallisation from methanol, and was found to be identical with a specimen obtained by direct condensation of acetone with hydantoin (C.P.S., 237, 3). Cyanomethylammonium cyanomethylthiolcarbamate (5 g.) and benzaldehyde (20 c.c.) were refluxed together for 1 hour, and the deep brown solution was cooled to 0° for 16 hours. Filtering and washing with methanol afforded colourless crystals of a *compound* (4·4 g.) which recrystallised from acetic acid in colourless rectangular plates, m. p. 225—226° (decomp.) (Found : C, 54·6; H, 5·2; N, 19·5. $C_{10}H_{11}ON_2S$ requires C, 54·3; H, 5·0; N, 19·0%). The compound was easily soluble in 10% aqueous sodium hydroxide to give a light orange solution, and with hot concentrated hydrochloric acid, a small quantity of an amorphous yellow powder was obtained.

Reactions with Amino-amides.—5-Imino-2 : 2-dimethyloxazolidine (30 g.) was added to warm water (30 c.c.) to give a solution of aminoacetamide (cf. Part III, *loc. cit.*). Ethanol (70 c.c.) was added to

the solution, and an excess of carbon oxysulphide (30 g.) was passed in during 5 hours. Colourless crystals of *carbamylmethylammonium carbamylmethylthiolcarbamate* (22 g., 80%) were filtered off, and crystallised from aqueous acetone in clusters of rectangular plates, m. p. 216—218° (decomp.) (Found: C, 29·2; H, 5·9; N, 26·8. $C_5H_{12}O_3N_4S$ requires C, 28·8; H, 5·8; N, 26·9%).

5-Imino-2 : 2-dimethyloxazolidine (11·5 g.) was added to warm water (12 c.c.). Potassium carbonate (7 g.) and ethanol (40 c.c.) were added, and an excess of carbon oxysulphide passed through the solution during 4—5 hours. Unchanged potassium carbonate (0·5 g.) was filtered off, the solution evaporated to 15—20 c.c., and acetone (20 c.c.) added. Two layers formed, and the lower layer gradually crystallised on standing at 0° to give massive rhombohedra of *potassium carbamylmethylthiolcarbamate* (12·5 g., 72%), recrystallising from water–50% methanolic acetone as large, colourless, hexagonal tablets, m. p. 194—195° (decomp.) (Found: C, 21·2; H, 3·0; N, 16·2. $C_3H_5O_2N_2SK$ requires C, 20·9; H, 2·9; N, 16·3%). This compound (5 g.) in water (50 c.c.) was shaken with benzyl chloride (5 c.c.) in ether (50 c.c.) for 40 hours. Colourless needles of *benzyl carbamylmethylthiolcarbamate* (4·55 g., 71%) were collected and recrystallised from water, toluene, or ethanol in glistening plates, m. p. 170—171° (Found: C, 53·3; H, 5·3; N, 12·9. $C_{10}H_{12}O_3N_2S$ requires C, 53·6; H, 5·4; N, 12·5%).

5-Imino-4-phenyl-2 : 2-dimethyloxazolidine (14 g.) was boiled for 5 minutes with water (20 c.c.) to give a solution of α-aminophenylacetamide in water. Ethanol (80 c.c.) and potassium carbonate (7·8 g.) were added, and an excess of carbon oxysulphide (25 g.) was passed through the solution during 4 hours. Unchanged potassium carbonate was separated, the solution evaporated to 15—20 c.c., and methanol (50 c.c.) and acetone (400 c.c.) added. A potassium salt, presumably potassium phenylcarbamylmethylthiolcarbamate (12·5 g., 68%), was filtered off and recrystallised from water–90% methanolic acetone. This salt was very hygroscopic, and it was difficult even to obtain an accurate m. p. which appeared to be 110—115°. The salt (8 g.) in water (20 c.c.) was shaken with benzyl chloride (6 g.) in ether (40 c.c.); the resulting colourless crystals of *benzyl phenylcarbamylmethylthiolcarbamate* (6 g., 62%) recrystallised from toluene in colourless felted needles, m. p. 150—151° (Found: N, 9·3. $C_{16}H_{16}O_3N_2S$ requires N, 9·3%).

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310. *The Enzymic Synthesis and Degradation of Starch. Part III. The Role of Carbohydrate Activators.*

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This paper is concerned with the function of carbohydrate activators in the synthesis of amylose by purified plant phosphorylase (P-enzyme). It lends support to the theory of Cori which pictures the synthesis as the growth of chain units from end-groups provided by the activator.

The activating powers of amylose and amylopectin have been compared, and the effects of (*a*) acid hydrolysis, (*b*) β-amylolysis, and (*c*) phosphorolysis on the activating power of each of these components of starch have been examined.

The conclusion is reached that potato P-enzyme functions by the endwise apposition of glucose residues (derived from glucose-1 phosphate) to a pre-existent chain (a "template chain") of 1 : 4-linked glucose members. The template chain may be an independent chain as in amylose, or an "outer" chain of a branched structure of the amylopectin type. It is shown that a template chain may consist of as few as 5—6 glucose members. Other workers, notably Weibull and Tiselius, have suggested that the minimum length of a template chain is three glucose units. Some evidence is also provided here that a template chain has an optimum, as well as a minimum length. This optimum length appears to be of the order of 20 glucose units.

The cross linkages of amylopectin are shown to constitute obstructions to the degradative function of P-enzyme as well as of β-amylase, and the striking inference can be drawn from this and previous work that none of the three enzymes examined, namely plant phosphorylase and the α- and β-amylases, is capable of inducing scission of the 1 : 6-glycosidic links of branched amylopectin structures.

ABUNDANT evidence is now available that the polysaccharide-synthesising enzymes of both animal and plant tissues only utilise glucose-1 phosphate (the Cori ester) as substrate if a preformed polysaccharide of the starch type is also present, and the original observation of Cori and Cori (*J. Biol. Chem.*, **1939**, **131**, 398) on muscle phosphorylase, and of Hanes (*Proc. Roy. Soc.*, 1940, *B*, **129**, 174) on potato phosphorylase (P-enzyme), have been fully substantiated by later investigations.

The work described in this paper makes a contribution to knowledge of the nature and function of such carbohydrate activators, and lends support to the hypothesis propounded by Cori *et al.* in 1943 (*J. Biol. Chem.*, **151**, 39). This hypothesis pictures the activator as providing end-groups which act as nuclei for the addition of new chain units, and was first proposed to explain the action of muscle phosphorylase but was later shown to be applicable also to potato phosphorylase. In a recent paper, Swanson and Cori (*J. Biol. Chem.*, **1948**, **172**, 815) have presented more detailed evidence based on the activating power of the hydrolytic products of

branched and unbranched polysaccharides with respect to the phosphorylases of muscle and of potato.

Our own approach to the problem has been along similar lines, but has been confined to the plant phosphorylase (P-enzyme). Our technique has been somewhat different from that of Cori, and the results are in some points interpreted differently: in essentials, however, our observations are in agreement with his.

Methods.—The power of a number of polysaccharides to " activate " the P-enzyme synthesis of amylose from glucose-1 phosphate was measured by a modification of the method of Green and Stumpf (*J. Biol. Chem.*, 1942, **142**, 355) which is based upon a determination of the amount of inorganic phosphate liberated in unit time by the action of P-enzyme on purified potassium glucose-1 phosphate. This compound was prepared from starch by the biological method. The activating power of a polysaccharide is given not in absolute units but relative to that of a standard. The standard chosen was a commercial soluble starch. With each P-enzyme preparation a control is carried out using 5 mg. of this soluble starch as primer, and the activating power of the polysaccharide (5 mg.) under test is given in terms of the activating power of soluble starch as unity. By this means it was possible to correlate the results with P-enzyme preparations of different activity.

The P-enzyme was prepared from potato juice and was purified by repeated precipitation with neutral ammonium sulphate solution (35%). It was free from Q-enzyme, amylases, and maltase.

The amylose and amylopectin specimens were prepared by one of the methods of fractionation described by Peat *et al.* in earlier papers. As criterion of degree of separation of the components of starch we take the " blue value " (for definition see experimental section) of the polysaccharide–iodine complex. The purest amylose prepared from potato starch has a blue value of 1·45, the purest amylopectin fraction from this starch having B.V., 0·15. Starch fractions of intermediate blue values are to be regarded as mixtures of these two components, and it is thus possible to calculate the proportion of each in a fraction of known intermediate B.V. The blue values and activating powers of a number of starch polysaccharides are given in Table 1.

TABLE I.

Activator in P-synthesis (5 mg. per 3·5 c.c. digest).	B.V. of activator.	Activating power relative to soluble starch.
Soluble starch (standard)	0·41	1·0
Potato starch	0·47	0·88
Amylose (aluminium hydroxide)	1·34	0·37
Amylose (thymol)	1·18	0·53
Amylopectin (thymol)	0·21	0·95
Amylopectin (*cyclo*hexanol–MeOH)	0·51	1·06
Amylopectin (*cyclo*hexanol)	0·18	1·02
Amylopectin treated with salivary amylase	0·09	1·28
Dextrin-A (from thymol-amylopectin)	0·19	0·10
Dextrin-A (from whole starch)	0·15	0·08
Dextrin-P (by P-enzyme on amylopectin)	0·19	0·65
Synthetic amylose I (maximum chain length)	1·33	0·23
Synthetic amylose II	0·40	1·05
Synthetic amylose III	0·27	1·19
Maltose	—	0

It is obvious from the results given in Table I that the two components of starch are markedly different with respect to their efficacy as activators in the synthesis of amylose by P-enzyme. At the concentrations employed, amylopectin is 2—3 times as active as amylose. Indeed, the purest sample of undegraded amylopectin (B.V., 0·15) is 2·9 times as active as the purest sample of amylose used (B.V., 1·34).

The higher the blue value of an amylose preparation, the smaller is its content of the branched-chain component, and it can be said with assurance that an amylose of B.V. 1·34 contains only a small proportion of amylopectin. It must be concluded therefore, since this amylose undoubtedly functions as an activator, that a branched structure is not essential for activation.

The difference in structure between amylose and amylopectin may be expressed as a

difference in the number of non-reducing chain ends per unit weight. Whereas in amylose one glucose unit in 200 (average) is a non-reducing end-group, in amylopectin the proportion is about 1 in 20. It would appear therefore that the activating power of a starch polysaccharide is primarily conditioned by the number of chain ends available in unit weight of the activator.

It is convenient, when speaking of the branched structure of amylopectin, to follow Myrbäck and refer to a chain between two branch points as an " inner " chain, an " outer " chain being that between a branch point and a non-reducing end. Limit dextrin-A (which is formed from amylopectin by β-amylolysis), although possessing the same number of terminal non-reducing glucose units per molecule as had the parent amylopectin, has lost the outer chains. The effect of this removal of the outer chains (average length, 10—12 glucose units) on the power to act as primer in the P-enzyme synthesis is illustrated in Table I. Dextrin-A was only one-tenth as active as the amylopectin from which it was derived, an indication that the inner chains (common to amylopectin and its derivative dextrin-A) are not nearly so effective in the activating mechanism as are the outer chains.

A number of bacterial polysaccharides including the dextran synthesised by *Leuconostoc dextranicum* were shown to have no activating power in the synthesis of starch by P-enzyme. Dextran is a polyglucose in which glucose residues are mutually joined mainly by 1 : 6-glucosidic linkages, and its failure, in the usual concentration, to act as a primer in starch synthesis demonstrates that in activating chains the glucose units must be linked as in maltose, *i.e.*, by α-1 : 4-glucosidic bonds. Proehl and Day (*J. Biol. Chem.*, 1946, **163**, 667) and Swanson and Cori (*loc. cit.*), however, find that bacterial dextran in high concentration has a slight activating power. On the other hand, Weibull and Tiselius (*Ark. Kemi, Min. Geol.*, 1945, **19***A*, No. 1) report dextran as being inactive.

The preliminary experiments recorded in Table I also give some indication of the effect of the length of unbranched chains on activator properties. The synthetic amylose I (B.V., 1·33) was prepared in a P-enzyme digest deficient in primer, *i.e.*, under conditions favourable for the production, according to Cori's theory, of chains of maximum length. The synthetic amyloses II and III resulted from syntheses in the presence of excess of primer and, although presumably unbranched, were of much shorter average chain-length than amylose I, as was shown by the respective blue values. Of these, the longest chain amylose (I) had the lowest activating power. Indeed, the activating power appeared to vary inversely as the blue value of the amylose.

Summarising, it is seen that to function as an activator (or primer) in the P-enzyme synthesis of amylose from glucose-1 phosphate, a carbohydrate must be a polyglucose of the starch type in which there are present, either as individuals or as part of a ramified structure, terminated chains of glucose residues joined by α-1 : 4-links. Amylopectin-free amylose is constituted entirely of such chains whereas only the " outer " chains of amylopectin conform to this description.

It was early realised that a factor of equal importance with the number of activating chains available was the actual length of an activating chain. It became apparent that chains containing a minimum number of α-1 : 4-linked glucose units constitute an essential feature of activator molecules. It will be convenient to refer to these terminated unbranched chains (for example, the " outer " chains of amylopectin) as template chains, and the function of P-enzyme is to be pictured as the extension of these template chains by the endwise apposition (by 1 : 4-linkage) of glucose units derived from glucose-1 phosphate. A template chain may be an individual chain (as in amylose) or only part of a longer chain (as in amylopectin). The proposition that an activating chain provides a " template " or " mould " for the phosphorylase brings into prominence the question of the minimum length of such a chain.

Weibull and Tiselius (*loc. cit.*) showed that independent unbranched chains of three, four, five, and six glucose units were capable of functioning as activators towards potato phosphorylase, and the evidence of Swanson and Cori (*loc. cit.*) on the activating power of acid-hydrolysed Schardinger dextrins confirmed that chains composed of only 4—5 glucose units could function as template chains for P-enzyme.

Knowledge of the part played by carbohydrate primers in starch synthesis has been extended by a study of the activating power of the products of hydrolysis of the separated components of starch. These products fell into three groups according as they were obtained from amylose and amylopectin by the agency of (*a*) acid, (*b*) β-amylase, and (*c*) P-enzyme.

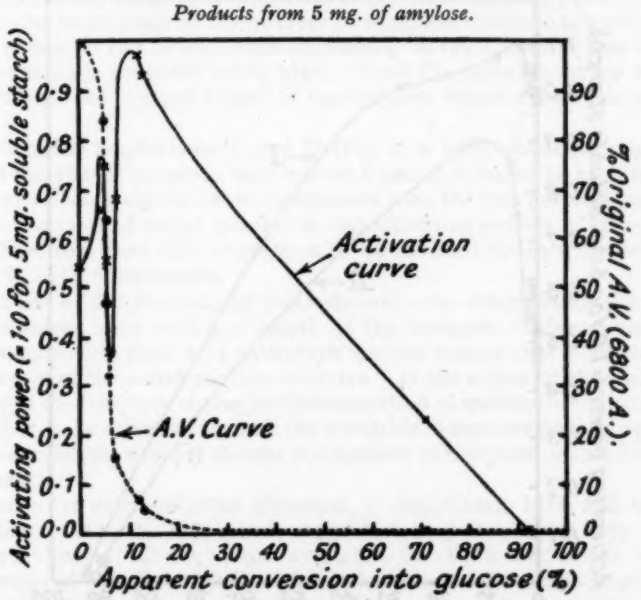
Activating Power of the Products of Acid Hydrolysis.—The course of hydrolysis of a starch polysaccharide by acid is two-fold. There occur (i) a rapid fragmentation of the polysaccharide chains into shorter-chain dextrins and (ii) the slower hydrolysis of these dextrins with the liberation of glucose.

(a) *Amylose.* The course of hydrolysis of thymol-amylose (B.V., 1·17) by sulphuric acid is shown by the " A.V.–conversion " curve of Fig. 1. The A.V.–conversion curve is that obtained when the absorption value (6800 A.) of the solution at a given stage in the hydrolysis is plotted (as a percentage of the original A.V.) against the reducing power (expressed as % conversion into glucose) at the same stage (see Bourne, Macey, and Peat, *J.*, 1945, 882). The initial rapid fall of A.V. (6800 A.), during which the blue coloration with iodine almost entirely disappears, represents the fragmentation into dextrins, to which the small increase in reducing power may be mainly ascribed. The second phase, the hydrolysis of dextrin to glucose, becomes predominant at the inflection point and is characterised by a rapid increase of reducing power with very little change in A.V. There is good reason to believe that the average chain-length of the dextrins at the inflection point is about 6. Dextrins of shorter chain-length than 6 are achroic.

FIG. 1.

The effect of acid hydrolysis on the activating power of amylose (B.V., 1·17).

Products from 5 mg. of amylose.



The P-enzyme activating power (relative to soluble starch) of a solution of amylose submitted to hydrolysis with hot N-sulphuric acid was determined at different stages of hydrolysis and plotted, also in Fig. 1, against % apparent conversion into glucose. It is seen that during the initial stages of hydrolysis the activating power of the solution increases rapidly with but little development of reducing power. An overall maximum, which is nearly twice the activating power of the original amylose solution, is attained at a stage when the reducing power of the solution is equivalent to an apparent conversion into glucose of only 10% and when the absorption value has fallen to about one-tenth of its original value. Two maxima are actually featured in Fig. 1. The relative activating power rises from 0·54 to the first maximum at 0·77, and then falls abruptly to 0·35. This fall coincides with the vertical part of the A.V.–conversion curve when the absorption value falls steeply with an almost negligible increase in reducing power. Thereafter the activation curve rises steeply to the second and higher peak at 10% conversion. Finally, the activating power diminishes continuously as the second phase of acid hydrolysis becomes predominant and the template chains are converted into non-activating glucose.

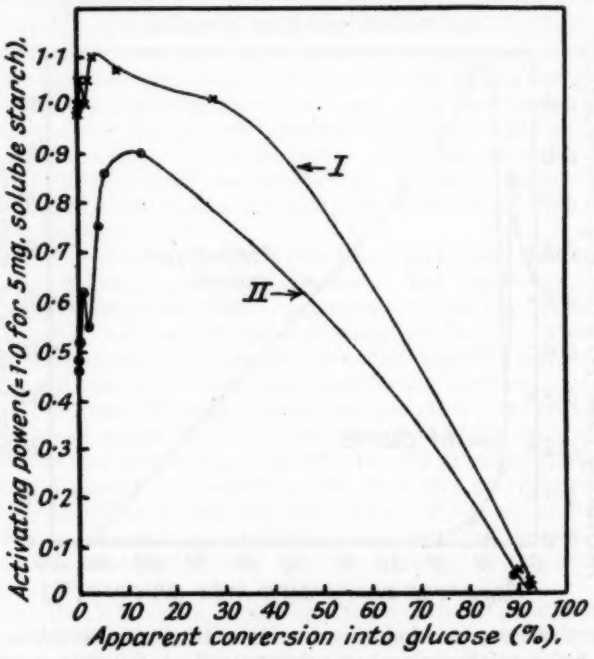
If it be assumed that, in the early stages, but little of the reducing power is due to the liberation of mono- or di-saccharides, then the minimum average length of the amylose fragments can be roughly calculated. Thus the achroic stage is reached at 20% apparent conversion into glucose, so that the average chain-length of the dextrins at this stage is 5 glucose units. This is in agreement with the commonly held view that dextrins of shorter chain-length than 6 or 7 give no colour with iodine. It should be noted that the achroic dextrins at 20% conversion still have a high activating power (0·8), and even at 80% conversion the activating power is still appreciable (0·14).

Swanson and Cori (*loc. cit.*, Fig. 1) observe a similar rise and fall in the activating power of acid-hydrolysed amylose. Only one peak is indicated, however, and this occurs at about 30% apparent conversion into glucose. The activating dextrins at this peak (glucose and maltose were removed by fermentation) were not more than 7 or less than 3 units long.

(b) *Amylopectin.* The effect of acid treatment on the activating power of amylopectin is shown in Fig. 2. This sample of amylopectin had B.V., 0·15, and contained a minimum of unbranched amylose impurity. Curve I (Fig. 2) shows that a small increase in activating power occurs in the earliest stage of hydrolysis, a peak being observed at about 5% apparent conversion into glucose. The curve is distinguished from that of amylose in the same concentration (see Fig. 1) by the much slower rate of fall of activating power as hydrolysis proceeds.

FIG. 2.

The effect of acid hydrolysis on the activating power of amylopectin (B.V., 0·15).



Curve I: Activation tests on products from 5 mg. of amylopectin.
„ II: „ „ „ 0·5 mg. „

It appeared that, in the region of maximum activation represented by the flattened peak of curve I, there existed in the solution a sufficiency of template chains (" outer " chains) to saturate the P-enzyme. After the attainment of maximum activating power, further increase in the number of template chains (by further acid fission) merely provided an excess. The experiment was therefore repeated with a solution of amylopectin only one-tenth as concentrated. Saturation of the P-enzyme was thus avoided, with the result that the activity peak was much sharper (curve II, Fig. 2).

It could be concluded from the sharpness of the amylose activation peak (Fig. 1) that in this case the number of template chains available was insufficient to saturate the P-enzyme at any stage in the hydrolysis, and that the real comparison between amylose and amylopectin is to be made with the amylopectin so diluted that the number of end groups (*i.e.*, template chains) is approximately the same as provided by the amylose. This is achieved when the weight ratio amylose/amylopectin is 10 : 1. The experiments of Swanson and Cori (*loc. cit.*) show a very slow change of activating power with degree of hydrolysis, and we would suggest that this may possibly be due to a saturation of the enzyme at the concentrations used.

There is a striking similarity of form between the activation curves of amylose (5 mg.) and amylopectin (0·5 mg.), a similarity which extends even to the display of two maxima (Fig. 1 and curve II, Fig. 2).

There will be two competing effects of the fragmentation of starch by acid hydrolysis: (i) an increase in the number of template chains, and (ii) a decrease in their average length. Factor (i) will operate immediately, and will increase the activating power, whereas factor (ii) will influence the activating power in the opposite sense by a shortening of the template chains to lengths below the minimum for activation. It is to be expected that factor (ii) will become effective at a later stage than (i). The result will be an initial rapid increase in activating power followed by a slower fall as the extent of hydrolysis increases.

The appearance of two maxima in each case is more difficult to understand. It would suggest that a third factor is involved, namely the existence of an optimum as well as a minimum length for a template chain. The first peak in the amylose curve (Fig. 1) is clearly defined and very sharp because, after the maximum has been attained, the activating power falls abruptly and this fall occurs with practically no increase in reducing power. In other words, the fall from the maximum activating power is coincident with the scission of long chains into shorter chains, the latter being sufficiently long, however, to have very little reducing power. The overall rise in activating power due to increase in the number of template chains is interrupted, on this view, by a fall which is due to the concomitant shortening of the chains to less than the optimum length. This optimum is probably fairly high. Since the reducing power at the first peak is 5% of that of glucose, the average length of the amylose fragments at this stage is at least 20 glucose units.

The first peak in the amylopectin Curve II (Fig. 2) is very small and might easily be overlooked. Were it not that it occurs in both curves I and II it might be ascribed to experimental error. It is in any case not significant in comparison with the first amylose peak. The essential fact is that there is a marked initial increase in the activating powers of acid-hydrolysed amylopectin and amylose, and that this increase is to be ascribed to an increase in the number of template chains in both components.

Activating Power of the Products of β-*Amylolysis.*—In determining the manner in which activating power might vary with the length of the template chains, β-amylolysis suggested itself as a better approach than acid hydrolysis for the reason that β-amylase does not bring about fragmentation of the substrate into dextrins. If the action of this enzyme results solely in the shortening of the template chains by the separation of maltose units from the non-reducing ends and if, further, it be assumed that all the available chains are equally open to attack, then it follows that β-amylolysis will not change the number of template chains but will diminish the average chain-length.

Certain evidence has been presented (Swanson, *J. Biol. Chem.*, 1948, **172**, 805; Cleveland and Kerr, *Cereal Chem.*, 1948, **25**, 139) which suggests that β-amylolysis may proceed, not by a simultaneous hydrolysis of all the chains available, but by a mechanism which involves the complete conversion of one chain into maltose before a second chain is attacked. The relative merits of these alternative hypotheses will be discussed in a later paper: in our opinion the facts of β-amylolysis are better accommodated by the first.

(a) *Amylopectin.* The variation of activating power with the extent of β-amylolysis of a solution of purified amylopectin (B.V., 0·15) is shown by curve I in Fig. 3. The activating power in general decreases rapidly as hydrolysis by β-amylase proceeds, and becomes very small when approximately half of the amylopectin has been converted into maltose. This agrees with the earlier observation that limit dextrin-A has very little activating power (Table I).

This general decrease in activating power is, however, preceded by a small rise (during the initial stage of β-amylolysis), a maximum being attained at about 5% conversion into maltose. This rise in activating power is made more obvious when the concentration of the original amylopectin solution is suitably diminished to avoid saturation of the P-enzyme by activator molecules (Curve II, Fig. 3).

(b) *Amylose.* The effect of β-amylolysis on the activating power of an amylose solution is also shown in Fig. 3. Curve III relates to a thymol-amylose of B.V. 1·07, which contained some of the branched component, and it is seen that the activating power of the amylose solution falls steadily with increasing hydrolysis until some 35% of the amylose has been converted into maltose. At this point there appears a marked arrest in the diminution of activating power which is maintained until 60% hydrolysis has occurred. Thereafter the activating power actually rises, and a maximum is attained at 80% conversion. Finally the curve falls away and the activating power becomes almost zero (at 92% conversion).

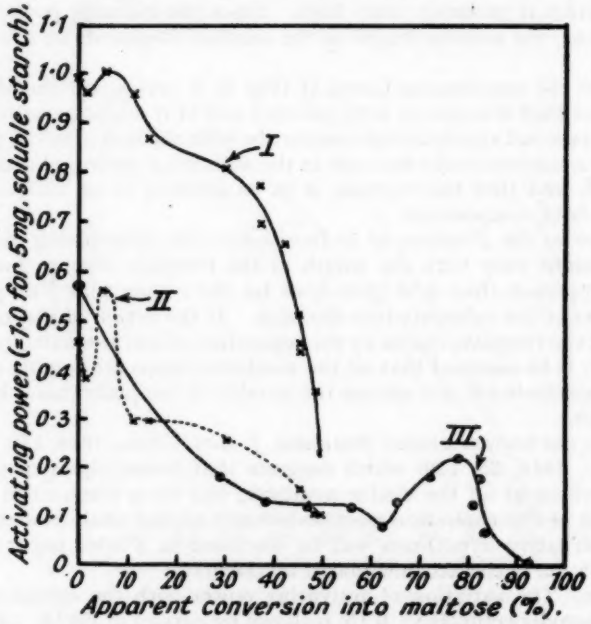
It is clear from this curve that a shortening of the amylose chain by the step-by-step removal of maltose from the reducing ends results in a diminution in P-enzyme activating power until a stage is reached represented by a conversion of 40—50% of the amylose into maltose. The

flattening of the curves could be ascribed to the existence of an optimum length of template chain which begins to be effective in the region of 40—50% conversion whereby an increase in activating power (as the average chain length approached the optimum) is superimposed upon the normal fall which would occur in the absence of an optimum.

On the basis of blue value it is calculated that an amylose of B.V. 1·07 contains not more than 30% amylopectin, and Curve I shows that this amylopectin would be almost inactivated by hydrolysis before the region of 40—50% conversion were reached. It can be accepted therefore that the activity peak at 80% conversion is mainly due to the unbranched chains of the amylose hydrolytic products. It can be calculated (when allowance is made for the presence of 20—30% amylopectin) that, if the chain-length of the original amylose is 200, the average chain length of the residual dextrins when 80% of the amylose (B.V., 1·07) has been converted into maltose will be 15—25 glucose units. This value agrees with that deduced from the acid hydrolysis curve (Fig. 1) for the optimum length of the template chain.

FIG. 3.

The effect of β-amylolysis on the activating power of amylose and amylopectin.



Curve I: Products from 5 mg. of amylopectin (B.V., 0·15).
„ II: „ „ 0·5 mg. „ „
„ III: „ „ 5 mg. of amylose (B.V., 1·07).

Phosphorolytic Degradation of Amylose and Amylopectin.—The phosphorolytic function of P-enzyme has not been so closely studied as has its synthetic function. The kinetics of the whole system, both synthetic and degradative, were examined by Hanes, and much data concerning phosphate equilibrium ratios were provided. The substrate used by Hanes was, however, whole starch, and it became desirable to investigate the phosphorolysis of the separated components.

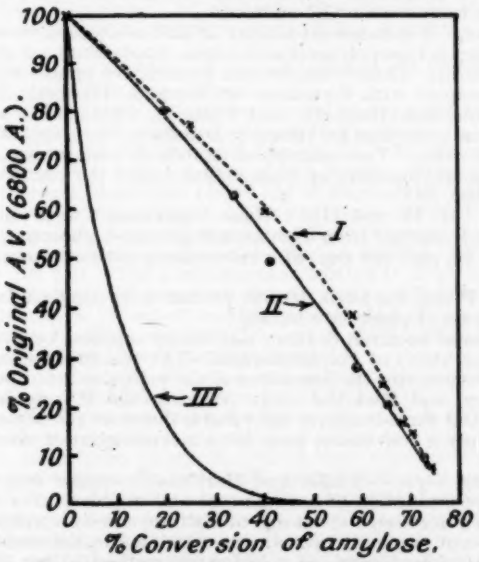
(a) *Amylose.* Repeated experiments established that P-enzyme preparations which were highly active in synthesis had no degradative action on amylose if phosphate ions were absent. In the presence of inorganic phosphate, however, amylose was readily converted by P-enzyme into glucose-1 phosphate. This phosphorolysis has many features in common with β-amylolysis of amylose. The progress of phosphorolysis was followed by the simultaneous estimation of % of original A.V. and % conversion into ester phosphate, and the A.V.–conversion curve compared with those for β-amylase and α-amylase (Fig. 4). The curves for P-enzyme (I) and β-amylase (II) are identical in form and almost coincidental. The amylose used in the two experiments had B.V. 1·07 and, as already stated, contained 20—30% amylopectin. The fact that, as shown by Fig. 4, the conversion of this amylose into glucose phosphate or maltose is not 100% may be explained as due to the presence of amylopectin in the above proportion.

Evidence is thus provided that degradation of amylose by P-enzyme proceeds by a mechanism similar to that of β-amylolysis, in that both methods of attack engage non-reducing chain-ends and split off single units (glucose phosphate or maltose) from these ends. α-Amylolysis follows a widely different course (see Curve III, Fig. 4).

(b) *Amylopectin.* It has been shown that P-enzyme catalyses the scission, as well as the formation, of α-1 : 4-linkages. Under certain conditions this enzyme effects the synthesis of unbranched chains but not of branched structures of the amylopectin type. P-Enzyme being incapable of establishing cross linkages, it is reasonable to suppose that neither can it catalyse the scission of such links. If this is so it can be inferred that complete phosphorolysis of natural amylopectin is not possible. Preliminary quantitative tests showed that amylopectin was partly degraded by P-enzyme, but only in the presence of inorganic phosphate. Consequently, in the later experiments, the digests all contained an adequate excess of phosphate to ensure the complete conversion, if it were possible, of the polysaccharide into glucose-1 phosphate.

FIG. 4.

The phosphorolysis and amylolysis of amylose (B.V., 1·07).



By (a) *P-Enzyme (to ester-phosphate).—Curve I.*
(b) *β-Amylase (to maltose).—Curve II.**
(c) *α-Amylase (to maltose).—Curve III.*†

* *Provided by Dr. W. J. Whelan.*
† *From Bourne, Macey, and Peat (J., 1945, 882).*

These experiments established that the cross-links in amylopectin obstruct P-enzyme in its degradative function in the same way as they act as a barrier to the further action of β-amylase. The arrest point is not, however, the same for the two enzyme systems. Whereas β-amylolysis ceased at 53% conversion (into maltose), P-enzyme carried the phosphorolysis to a limit of 39% conversion (into glucose phosphate). The limit dextrins (named in this paper dextrin-A and dextrin-P respectively) both gave red stains with iodine and showed the same blue value (0·19).

It was further shown that the higher degree of hydrolysis effected by the β-amylase was not due to the contamination of the latter enzyme with α-amylase inasmuch as the dextrin-A was not susceptible to attack by β-amylase whereas the dextrin-P was. β-Amylolysis of dextrin-P brought about a further conversion of 18% (based on weight of original amylopectin) into maltose.

It is clear that phosphorolytic degradation does not shorten the outer chains of amylopectin to the same extent as does β-amylolysis. Indeed, at 40% conversion, when P-enzyme action ceases, the shortened outer chains are still long enough to function as templates for the synthetic action of P-enzyme. This is shown by the fact that dextrin-P is 6—7 times more active as a primer than is dextrin-A (see Table I).

The approximate length of the template chains of dextrin-P can be calculated from the conversion figures. Accepting 20 as the average number of glucose residues in the repeating chain unit of amylopectin, it follows from the fact that dextrin-P is converted into dextrin-A by β-amylase with the concomitant liberation of 18% by weight of the original amylopectin as maltose that the repeating chains of dextrin-P are, on the average, longer than those of dextrin-A by 3—4 glucose units. What may be called the outer chains of dextrin-A are composed of at least one, and possibly two, glucose members (Haworth, Kitchen, and Peat, *J.*, 1943, 619), so that the outer chains of dextrin-P contain at least 5—6 glucose units. Further evidence is thus afforded that a chain of as few as 6 units will function as a template chain.

Experimental.

Sources of the Enzymes.—The salivary α-amylase employed in this work was prepared by the procedure of Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924), and was free from maltase. The β-amylase, isolated from soya beans, showed neither α-amylase nor maltase activity (see Bourne, Macey, and Peat, *loc. cit.*). The preparation of amylase-free potato phosphorylase (P-enzyme) has been described earlier (Bourne and Peat, *J.*, 1945, 877).

Sources of the Carbohydrate Activators.—(a) *Maltose.* This was a highly purified specimen obtained by fractional crystallisation from commercial maltose.

(b) *Acid-solubilised starch.* A commercial sample of acid-solubilised starch was used as a standard primer for the synthesis of starch-type polysaccharides from dipotassium α-D-glucopyranose-1 phosphate.

(c) *Amylose and amylopectin.* These were derived from native potato starch by selective precipitation of the amylose as a complex with thymol or *cyclo*hexanol (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19; Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687) and by adsorption of the amylopectin on an aluminium hydroxide gel (Bourne, Donnison, Peat, and Whelan, *J.*, 1949, 1).

(d) *Dextrin-A* (*α-amylodextrin*). Two samples of dextrin-A were prepared, one from native potato starch and one from thymol-amylopectin, by β-amylolysis under the conditions prescribed by Bourne, Haworth, Macey, and Peat (*loc. cit.*).

(e) *Synthetic "amyloses"* (I, II, and III). These "amyloses" were unbranched polysaccharides synthesised by amylase-free P-enzyme from dipotassium glucose-1 phosphate (220 mg.) in the presence of varying proportions (0·5, 60, and 120 mg.) of a red-staining activator prepared from potato amylose by acidic hydrolysis.

(f) *Dextrin-P.* Dextrin-P was the limit dextrin produced by amylase-free P-enzyme from amylopectin in the presence of excess of phosphate buffer.

(g) *Dextran.* The sample of bacterial dextran was kindly supplied by Professor M. Stacey.

Measurement of the Iodine Stains of Polysaccharides.—The two expressions, absorption value (A.V.) and blue value (B.V.), used to describe the intensities of the iodine stains of polysaccharides were defined by Bourne, Haworth, Macey, and Peat (*loc. cit.*). Whereas the B.V. is determined under standard conditions of concentration and wave-length of light and is therefore characteristic of the polysaccharide in question, the A.V. is simply a convenient term for a measurement of relative intensity made under any conditions.

Determination of Reducing Sugar.—Shaffer and Hartmann's copper reagent (*J. Biol. Chem.*, 1921, **45**, 377) was used for the determination of both glucose and maltose. For the determination of very small amounts ($< 0·2$ mg. of glucose and $< 0·4$ mg. of maltose) a known weight of the appropriate sugar was introduced, and the amount originally present was calculated by difference.

Determination of Inorganic Phosphorus.—A colorimetric method (Allen, *Biochem. J.*, 1940, **34**, 858) was used for the determination of inorganic phosphorus. Calibration of the reagents against potassium dihydrogen phosphate gave a linear graph when the absorption value measured on a Spekker absorptiometer was plotted against the weight of phosphorus present. This graph showed the method to be adequate for the determination of 0·02—0·20 mg. of inorganic phosphorus.

Standard Procedure for the Measurement of the Activating Power of a Carbohydrate.—A modification of the method employed by Green and Stumpf (*loc. cit.*) for the determination of phosphorylase activity was used for the measurement of the activating power of a carbohydrate. A standard digest containing citrate buffer (pH 6·0; 1·0 c.c.), dipotassium glucose-1 phosphate (0·1M; 1·0 c.c.), the activator solution (1·0 c.c., containing 5 mg. of carbohydrate), and P-enzyme solution (0·5 c.c.), the last being added to the remainder of the digest only when the temperature had reached 35·5°, was incubated at 35·5° for 12 minutes. The synthesis was arrested with 6% trichloroacetic acid solution (5 c.c.), the protein precipitate removed on the centrifuge, and an aliquot portion of the supernatant liquid analysed colorimetrically for inorganic phosphorus. A simultaneous control digest, containing the same solutions except that the activator solution was replaced by an equal volume of water, served to measure the extent to which the synthesis had been primed by natural activators present in the glucose-1 phosphate, which had been prepared enzymically from potato starch. The synthesis achieved in this control was always small in comparison with that in the main digest. The increase in inorganic phosphorus attributable to the carbohydrate under test was found by difference.

The activating power of the carbohydrate primer relative to that of commercial soluble starch, determined under the same conditions, is given as the ratio of the weight of phosphorus liberated in the presence of 5 mg. of carbohydrate to the weight liberated in the presence of 5 mg. of soluble starch.

Effect of Acid Hydrolysis on the Activating Power of Amylose.—Thymol-amylose (500 mg.; B.V. 1·17) was dissolved in hot water (45 c.c.) containing a little sodium hydroxide. The solution was cooled, neutralised with sulphuric acid, diluted to 50 c.c., and mixed with 2N-sulphuric acid (50 c.c.). It was placed under a reflux condenser in a water-bath at 75°, and the temperature of the bath was then slowly raised to 100°. In this way the initial stages of the hydrolysis could be studied more closely than would have been possible had the reaction been started at 100°. At selected stages of the reaction an aliquot

volume (5 c.c.) was removed and adjusted to *ca.* pH 6·0 with sodium hydroxide, the final volume being 10 c.c. The following analyses were made on each neutral sample :

(*a*) A portion (2 c.c. ≡ 5 mg. of amylose) was added to water (200 c.c.), acidified with 6N-hydrochloric acid (3 drops), stained with a solution (5 c.c.) of 0·2% iodine in 2% potassium iodide, and diluted to 500 c.c. The A.V. (6800 A.) of this coloured solution was determined.

(*b*) The reducing power of a second portion (2 c.c. ≡ 5 mg. of amylose) was measured by the Shaffer–Hartmann method.

(*c*) A third portion (2 c.c. ≡ 5 mg. of amylose) was tested as an activator of the synthetic function of P-enzyme. Since only 1 c.c. of activator solution was normally used in the standard method it was necessary to dissolve the glucose-1 phosphate directly in the citrate buffer to preserve the usual concentrations.

The results of this experiment are shown graphically in Fig. 1.

Effect of Acid Hydrolysis on the Activating Power of Amylopectin.—An amylopectin sample (B.V., 0·15), prepared from potato starch by the *cyclo*hexanol–methyl alcohol method, was hydrolysed with acid under the conditions which have already been described in the case of amylose. In a duplicate experiment a ten-fold dilution (≡ 0·5 mg. of amylopectin) of the neutralised hydrolysate was used in the activation tests. The results are recorded in Fig. 2.

Effect of β-Amylolysis on the Activating Power of Amylose.—A solution of thymol-amylose (500 mg.; B.V., 1·07) in water containing a little sodium hydroxide was neutralised with sulphuric acid, diluted to 50 c.c., and mixed with citrate buffer (pH 6·0; 25 c.c.) before being incubated at 36° with a 0·3% solution (25 c.c.) of soya-bean β-amylase. At intervals, aliquot volumes (5 c.c.) of the digest were withdrawn, inactivated by immersion in a boiling water-bath for 4 minutes, and used for the following measurements :

(*a*) A portion (1·0 c.c. ≡ 5 mg. of amylose) was subjected to the Shaffer–Hartmann determination, the reducing power being expressed in terms of maltose.

(*b*) A portion (1·0 c.c. ≡ 5 mg. of amylose) was tested as a primer for the synthetic action of P-enzyme by the standard method. The results are shown in Fig. 3.

Effect of β-Amylolysis on the Activating Power of Amylopectin.—The previous experiment was repeated on amylopectin (B.V., 0·15) prepared from potato starch by the *cyclo*hexanol–methyl alcohol method. In a duplicate experiment a ten-fold dilution (≡ 0·5 mg. of amylopectin) of each inactivated test sample was used for the activating power measurement. The results are recorded in Fig. 3.

Phosphorolysis of Amylose.—A solution of thymol-amylose (96 mg.; B.V., 1·07) in water containing a little sodium hydroxide was neutralised with sulphuric acid, diluted to 40 c.c., and mixed with a 0·2M-phosphate buffer (40 c.c.; pH 6·4). The solution was incubated at 35·5° with an amylase-free P-enzyme solution (16 c.c.), aliquot volumes being removed at intervals for the following measurements :

(*a*) A digest sample (1·0 c.c. ≡ 1·0 mg. of amylose) was diluted with water (50 c.c.), slightly acidified with hydrochloric acid, stained with a solution (1·0 c.c.) of 0·2% iodine in 2% potassium iodide, and diluted to 100 c.c. The A.V. (6800 A.) was then measured.

(*b*) The reducing power of a sample (5·0 c.c.) was determined by the Shaffer–Hartmann method. No reducing sugars were formed throughout the period of digestion.

(*c*) A portion (5 c.c.) of the digest was boiled for 30 seconds, cooled, and mixed with a solution (5 c.c.) of magnesium acetate (0·09 g.). Ammonia solution was added to precipitate the bulk of the inorganic phosphate as magnesium ammonium phosphate, which was removed by filtration and washed with water. The filtrate and washings were diluted with water to 100 c.c., and determinations of inorganic and total phosphorus were made on this solution. The amount of ester phosphorus was calculated by difference. Control experiments on known mixtures of glucose-1 phosphate and the phosphate buffer in proportions similar to those present at different stages in the above digest showed that the ester phosphorus determination was correct to within 2%. The results are shown graphically in Fig. 4.

Phosphorolysis of Amylopectin.—A digest containing a dispersion of amylopectin (180 mg.; B.V., 0·15) in water (90 c.c.), phosphate buffer (0·2M; pH 6·4; 75 c.c.), and amylase-free P-enzyme solution (15 c.c.) was incubated at 35·5°. At intervals the reducing power and ester phosphorus were determined by the methods used in the previous digest. The results are given in Table II.

TABLE II.

Phosphorolysis of Amylopectin (*B.V.*, 0·15).

Time of incubation (min.).	Apparent conversion (%) into maltose (from R_{Cu}).	Conversion (%) of amylopectin into ester phosphate.
0·5	Nil	0·0
30	,,	29·8
126	,,	39·7
260	,,	39·2
325	,,	38·7

After 325 minutes the digest was inactivated by boiling, and cooled. An aliquot portion (10 c.c.) was incubated at 35·5° with a 0·25% solution (10 c.c.) of soya-bean β-amylase, and the reducing power was determined at intervals by the Shaffer–Hartmann method. The conversion into maltose, calculated on the original amylopectin, was 12·5% (1 hour) and 18·1% (22 hours).

The authors wish to express their thanks to Professor Sir Norman Haworth, F.R.S., for his interest, and to the Colonial Products Research Council for financial aid to one of them (D. A. S.). They are grateful also for the assistance of Mr. S. A. Barker, a member of this research group.

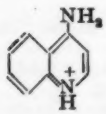
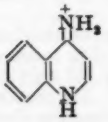
THE UNIVERSITY, EDGBASTON, BIRMINGHAM, 15. [*Received, August 19th*, 1948.

311. *Synthetic Antimalarials. Part XLI. Physicochemical Studies on Quinoline Derivatives.*

By J. C. Gage.

The absorption spectra and dissociation constants of a number of 4-aminoquinoline derivatives have been measured. The influence of substituents on these properties is correlated with their effect on antimalarial activity, and it is shown that the activity is closely connected with the distribution of cationic charge between the 4-amino- and heterocyclic-nitrogen atoms; a similar charge distribution can be identified in a number of chemical types which show a specific antimalarial activity.

The structure of the 4-aminoquinoline ion has been discussed by Albert and Goldacre (*Nature*, 1944, **153**, 467), Drake, Creech, Draper, Garman, Haywood, Peck, Walton, and Van Hook (*J. Amer. Chem. Soc.*, 1946, **68**, 1214), and by Irvin and Irvin (*J. Amer. Chem. Soc.*, 1947, **69**, 1091); it has been suggested that the proton is attached to the heterocyclic nitrogen atom and that the ion is stabilised by resonance of the type (I*a*, *b*), which involves a distribution of the charge between the two nitrogen atoms.

(I*a*.)   (I*b*.)

Quinoline derivatives carrying a dialkylaminoalkylamino-group or basic side-chain in the 4-position form a well-known group of drugs with a high specific antimalarial activity. The ultra-violet absorption spectra and pK values of certain members of this series were studied by Irvin and Irvin (*loc. cit.*). The present communication extends the investigation of these authors, and the findings are discussed with reference to Parts XXXIV and XXXVI of this series (Gage, this vol., pp. 221, 469).

The Calculation of Dissociation Constants.

In Part XXXVI (Gage, *loc. cit.*) equations (1) and (2) were derived for calculating the dissociation exponents (pK values) of an unsymmetrical diacid base by potentiometric titration, where K_1 and K_1' are the constants of the equilibria between the base B_1B_2 and protons to form the ions $H^+B_1B_2$ and $B_1B_2H^+$, respectively (B_1 being the more basic centre), and K_2 the constant of the equilibrium of $H^+B_1B_2$ and a proton; y and pH are quantities determined from the titration. The exponents of 4-aminoquinoline and the aliphatic diamines (Tables I and II), from which an approximation to K_1/K_1' can be obtained, indicate that in the dialkylamino-alkylaminoquinolines this term may not be negligible. It is not possible to derive K_1/K_1' from titration data, but, as indicated below, it may be determined from the spectrometric results.

$$pK_1 = pH - \log_{10}[(1-y)/y] - \log_{10}(1 + K_1/K_1') \quad . \quad . \quad . \quad . \quad (1)$$

$$pK_2 = pH + \log_{10}[(2-y)/(y-1)] + \log_{10}(1 + K_1/K_1') \quad . \quad . \quad . \quad (2)$$

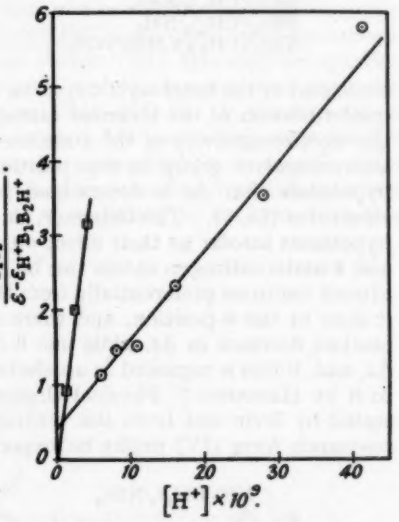
The difference between the first and second dissociation constants of the basic-side-chain pyrimidines examined in Part XXXVI (Gage, *loc. cit.*) permitted them to be regarded as monoacid bases for the purpose of deriving K_2 from spectrometric observations, but the probable value of K_1/K_1' in the quinolines containing a basic side-chain at position 4 suggests that this treatment may not justifiably be extended to this series. It can readily be shown that K_2 may be calculated by equation (3) from the molar extinction coefficient ε of a solution at hydrogen-ion concentration $[H^+]$, provided that the coefficients of the four components of the equilibrium of the base B_1B_2 in the presence of hydrogen ions ($\varepsilon_{B_1B_2}$, $\varepsilon_{H^+B_1B_2}$, $\varepsilon_{B_1B_2H^+}$, $\varepsilon_{H^+B_1B_2H^+}$) are known. If $[H^+]$ is selected so that $K_1'/[H^+]$ is negligible, and if it is assumed, as by Irvin and Irvin, that $\varepsilon_{B_1B_2}$ equals $\varepsilon_{H^+B_1B_2}$, and $\varepsilon_{B_1B_2H^+}$ equals $\varepsilon_{H^+B_1B_2H^+}$, then (3) reduces to (4).

$$[H^+]/K_2 = (\varepsilon_{H^+B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+}) + [(\varepsilon_{B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+})]K_1/[H^+] + [(\varepsilon_{B_1B_2H^+} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+})]K_1/K_1' \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$[H^+]/K_2 = (\varepsilon_{B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+}) - K_1/K_1' \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

A plot of $[H^+]$ against $(\varepsilon_{B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+})$ should yield a straight line, the slope of which gives K_2, and the intercept when $[H^+]$ equals zero will be K_1/K_1'. It is shown later that the

absorption spectrum of the ionised 4-aminoquinoline system is influenced by the electron affinity of the 4-amino-substituent, and the considerable electronegative inductive effect of the charged β-diethylaminoethyl group (Gage, Part XXXVI, *loc. cit.*) suggests that in 4-β-diethylaminoethylaminoquinoline the assumption that $\varepsilon_{B_1B_2H^+}$ equals $\varepsilon_{H^+B_1B_2H^+}$ may not be justifiable. The figure, however, shows that with this compound the plot of $[H^+]$ against $(\varepsilon_{B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{H^+B_1B_2H^+})$ is almost a straight line, which suggests that any deviations from the assumptions on which (4) is based are of no great significance.



⊙ *4-β-Diethylaminoethylaminoquinoline.*
⊡ *4-γ-Diethylaminopropylaminoquinoline.*

Discussion.

The results in Table I show that the dissociation exponent of the heterocyclic system (pK_2) of the 4-basic side chain quinolines (II) increases as the chain length increases; this may be attributed to the decrease in the electronegativity of the charged substituent on the 4-amino-group, and some measure of this inductive effect may be obtained from the difference between the pK_1 and pK_2 values (ΔpK) of the corresponding alkylenediamines (Table II).

In weakly acid solution the absorption spectra of 4-aminoquinoline derivatives show an absorption band with a double peak within the range 325 to 350 mμ. which is closely associated with the existence of the resonance forms (I*a*, *b*) (Drake *et al.*, *loc. cit.*; Irvin and Irvin, *loc. cit.*). Table I shows the effect of substituents on the 4-amino-group on the difference between the extinction coefficients of these two peaks ($\Delta\varepsilon$). Since the basic side-chain is saturated its influence on the absorption spectrum must be attributed to its inductive effect; the alternative assumption, that there is an interaction between the terminal nitrogen atom of the chain and

TABLE I.

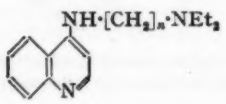
The antimalarial activities were communicated privately by Dr. D. G. Davey. The detailed results will be published later; the methods of assessing activity and reporting the results are referred to in Part I (Curd and Rose, *J.*, 1946, 343). Experimental details of the spectrometric and titrimetric determinations are given in Part XXXVI (*loc. cit.*).

Quinoline substituents.	$\varepsilon_{max.}$ at λλ given in parentheses.		Δε.	p*K*. Titrimetric, pK_1.	p*K*. Titrimetric, pK_2.	p*K*. Spectrometric, pK_2.	Antimalarial activity. Dose mg./kg.	Activity.
4-Amino	12,450(320)	10,900(335)	1550					
4-Methylamino	15,100(325)	15,050(338)	50					
4-Methylamino-6-methoxy	11,800(338)	12,200(350)	−400					
7-Chloro-4-methylamino	15,000(328)	15,500(340)	−500					
4-β-Diethylaminoethylamino-6-methoxy	10,650(338)	10,700(347)	− 50				80	++
							40	+ to ++
							20	+
7-Chloro-4-β-diethylaminoethylamino	15,150(325)	14,400(337)	750				10	++
							5	±
6-Chloro-4-γ-diethylaminopropylamino	14,400(334)	14,200(346)	200				40	++
							20	(?)
7-Chloro-4-γ-diethylaminopropylamino	16,150(328)	16,350(340)	−200				5	++
							2	±
4-β-Diethylaminoethylamino	16,300(325)	15,000(338)	1300	8·95	7·90	8·10	80	++
							40	+ to ++
							20	+
4-γ-Diethylaminopropylamino	16,500(326)	16,000(337)	500	10·00	8·55	8·75	10	++
							8	+
4-δ-Diethylaminobutylamino	15,700(327)	15,800(340)	−100	10·25	8·80	9·10	20	++
							10	+
4-δ-Diethylamino-α-methylbutylamino	17,100(328)	17,500(340)	−400	10·25	8·85	9·05	20	++
							10	+
							5	±
4-3′-Diethylaminopropylamino 3′-methiodide	16,650(326)	15,850(337)	800	—	8·55	8·65	—	—

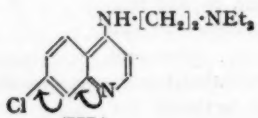
TABLE II.

Compound.	pK_1.	pK_2.	ΔpK ($= \log_{10} 1/E$).
$NEt_2 \cdot [CH_2]_2 \cdot NEt_2$	9·3	6·65	2·65
$NH_2 \cdot [CH_2]_2 \cdot NH_2$	9·8	8·8	1·0
$NEt_2 \cdot [CH_2]_3 \cdot NEt_2$	9·75	8·60	1·15
$NEt_2 \cdot [CH_2]_4 \cdot NH_2$	10·3	9·2	1·1
$NEt_2 \cdot [CH_2]_3 \cdot CHMe \cdot NH_2$	10·1	9·55	0·55

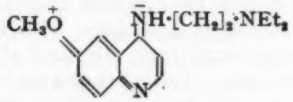
some part of the heterocyclic system, is improbable in the doubly charged cation, and, moreover, quaternisation of the terminal nitrogen does not significantly affect $\Delta\varepsilon$ or pK. A decrease in the electronegativity of the 4-amino-substituent is attended by a decrease in $\Delta\varepsilon$, and, since an electronegative group in this position will favour the resonance form (I*b*), it is a reasonable hypothesis that $\Delta\varepsilon$ is determined to some extent by the relative contributions of the two structures (I*a*, *b*). The influence, on $\Delta\varepsilon$, of substituents on the quinoline nucleus supports the hypothesis insofar as their effect on the electron-density distribution between the heterocyclic and 4-amino-nitrogen atoms can be predicted; chlorine in the 7-position might be expected to attract electrons preferentially from the heterocyclic nitrogen atom (III) to a greater extent than it does in the 6-position, and there are three examples of 7-chloro-compounds which show a marked decrease in $\Delta\varepsilon$, while one 6-chloro-derivative does not. A 6-methoxy-group decreases $\Delta\varepsilon$, and, if this is regarded as an electron-donating group in conformity with the σ value ascribed to it by Hammett (" Physical Organic Chemistry ", New York, 1940, p. 188), rather than, as stated by Irvin and Irvin (*loc. cit.*), as an electron-attracting group, then the possibility of the resonance form (IV) might be expected to decrease the contribution of (I*a*) in the ion.



(II.)



(III.)

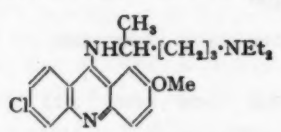


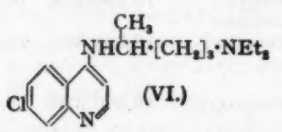
(IV.)

The antimalarial activities recorded in Table I indicate that 4-β-diethylaminoethylamino-quinoline (II; $n = 2$) has an appreciably lower biological activity than its higher homologues; this cannot be attributed entirely to the effect of the length of the basic side-chain on pK_2 since the activity reappears in the 7-chloro-derivative. The pK_2 of this latter compound has not been accurately determined, but a potentiometric-titration curve has shown that it is not higher than that of (II; $n = 2$). A 7-chloro-group also increases the biological activity of (II; $n = 3$), but there is evidence from the 6-chloro-derivative of this compound that chlorine in this position has a dystherapeutic effect, though the biological results available at present are not sufficiently conclusive for this to be definitely established. The above observations suggest that there is some parallel between the effect of substituents on $\Delta\varepsilon$ and their influence on antimalarial activity. According to this hypothesis, 4-β-*diethylaminoethylamino*-6-*methoxyquinoline* should possess a higher antimalarial activity than does (II; $n = 2$); this is not evident in the biological results. The active antimalarial mepacrine (V) does, however, contain a methoxy-group in an analogous position relative to the heterocyclic and amino-nitrogen atoms, in addition to a chlorine atom in a position analogous to that in which it occurs in the 7-chloroquinolines. Both (V) and the antimalarial " Chloroquine " (VI) contain the δ-diethylamino-α-methylbutyl group in the basic side-chain; this group produces the lowest ΔpK value of the diamines recorded in Table II, and it also gives the lowest value for $\Delta\varepsilon$ in the series (II).

The analogy between (V) and (VI) is very close; it can be extended to include a number of other chemical types which show a specific antimalarial activity, and the diagram (VII;

(V.)

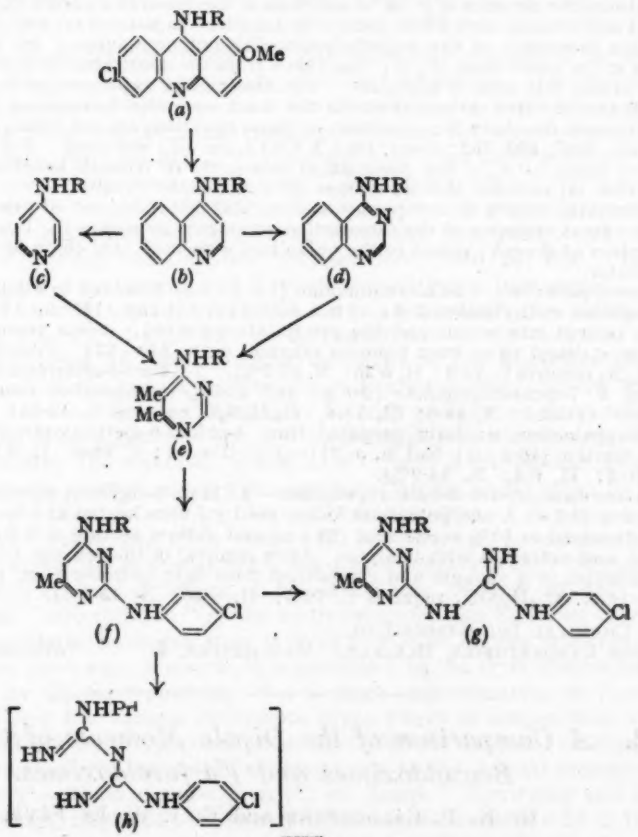




(VI.)

R = dialkylaminoalkyl) shows the family relationships which exist between the acridine, quinoline, quinazoline, pyridine, and pyrimidine antimalarials. It will be seen that the system consisting of a substituted amino-group *para* to a heterocyclic nitrogen atom (VIII) is common to each of these chemical types. The recorded dissociation exponents of mepacrine (Christophers, *Ann. Trop. Med. Parasitol.*, 1937, **31**, 43), 4-aminopyridine (Albert and Goldacre,

loc. cit.), the pyrimidine derivatives (Gage, Part XXXVI, *loc. cit.*), and the 4-aminoquinolines (Table I) indicate that in these compounds the heterocyclic system exists largely as the ion at the pH of biological fluids. pK_a of the quinazoline derivative (VII*d*; R = $NEt_2{\cdot}[CH_2]_2$) has been found by potentiometric titration to be 7·6 and is therefore of the same order as that of the pyrimidines (VII*e* and *f*). It is possible, therefore, that the essential pharmaco-dynamic group of all these chemical types comprises an amino-nitrogen atom *para* to a protonised heterocyclic nitrogen atom, both of which share the cationic charge (IX); this may be regarded as an extension of Schönhöfer's suggestion that antimalarial activity is connected with the tautomeric possibilities of the group (VIII) (*Z. physiol. Chem.*, 1942, **274**, 1). If the hypothesis that $\Delta\varepsilon$ depends on the electron-distribution of (IX) be accepted, the parallelism between the effect of substituents on the biological activity of the quinolines containing a basic side-chain and their effect on $\Delta\varepsilon$ indicates that an important function of the remainder of the molecule may be suitably to regulate this electron distribution. It is necessary to exclude from this



(VII).

generalisation the peculiar property of the charged terminal nitrogen atom of the basic side-chain, which, as suggested by Delektorskaya and Lipowitsch (*Arch. Pharm.*, 1934, **272**, 24), is most probably conductophoric and which appears to be entirely divorced from the function of the group (IX); although, since both parts of the molecule are essential for specific antimalarial activity, some limit to their spatial relationship may be required.

The above hypothesis does not exclude the existence of structures other than (IX) with a high potential antimalarial activity; there are notable omissions from scheme (VII), for example, quinine, the quinoline carbinols, and pamaquin; the first two of these do not possess the amino-nitrogen of (IX), and in none is the heterocyclic system appreciably ionised at physiological pH values. The inclusion of the diguanide antimalarials (VII*h*) may be criticised as the structure of the ion has not been elucidated (Gage, Part XXXIV, *loc. cit.*); nevertheless, it is evident that a structure analogous to (IX) may also exist in this chemical type.

Experimental.

The molar extinction coefficients were determined as described in Part XXXVI (Gage, *loc. cit.*). The figure shows the variation of $(\varepsilon_{B_1B_2} - \varepsilon)/(\varepsilon - \varepsilon_{HB_1B_2H^+})$ with $[H^+]$ for 4-β-diethylaminoethylaminoquinoline and 4-γ-diethylaminopropylaminoquinoline; the value of K_2 and K_1/K_1' for the former may be calculated from the slope of the line and the intercept by equation (4); the intercept and, therefore, K_1/K_1' of the latter compound is negligible.

For potentiometric titration 4×10^{-4}M-solutions of the bases in a known excess of hydrochloric acid were prepared and titrated with 0·01N-sodium hydroxide. Equations (1) and (2) were used to calculate the dissociation exponents of the 4-diethylaminoalkylaminoquinolines; for the derivative with two carbon atoms in the alkyl chain K_1/K_1' was taken from the spectrometric results, whilst with three or more carbon atoms this term is negligible. The dissociation exponents of the symmetrical alkylenediamines with two or three carbon atoms in the chain were also determined by (1) and (2), but with four carbon atoms in the chain the conditions of these equations are not fulfilled, and Barton's equation (Barton, *Nature*, 1947, **160**, 752; Gage, Part XXXVI, *loc. cit.*) was used. For this calculation, K_1 was assumed to be equal to K_1'; the dissociation exponents of trimethylenediamine and its tetraethyl derivative (Table II) indicate that no serious error is thereby involved.

The experimental results do not permit a statistical determination of precision, but there is little doubt that the great majority of the dissociation exponents lie within the limits ±0·05 unit, with the possible exception of the pK_1 values of the quinolines with basic side-chain for which the error may be somewhat greater.

4-Methylaminoquinolines.—4-Chloroquinoline (1·0 g.) was dissolved in ethanol (15 c.c.) and treated with 21·5% aqueous methylamine (3·0 c.c.) in a sealed tube at 155—165° for 12 hours. *4-Methylaminoquinoline* was poured into water, and the precipitate collected; it was treated with charcoal in hot ethanol and crystallised twice from aqueous ethanol, m. p. 230—231° (Found: C, 75·5; H, 6·1; N, 17·35. $C_{10}H_{10}N_2$ requires C, 75·9; H, 6·35; N, 17·7%). *7-Chloro-4-methylaminoquinoline* was similarly prepared from 4 : 7-dichloroquinoline (2·0 g.) and 21·5% methylamine solution (5·0 c.c.) and had m. p. 247—248° (Found: N, 14·6; Cl, 18·8. $C_{10}H_9N_2Cl$ requires N, 14·55; Cl, 18·85%). *4-Methylamino-6-methoxyquinoline*, similarly prepared from 4-chloro-6-methoxyquinoline (19 g.) and 21·5% methylamine solution (46·5 c.c.), had m. p. 211—212° (Found: C, 69·8; H, 6·3; N, 15·3. $C_{11}H_{12}ON_2$ requires C, 70·2; H, 6·4; N, 14·9%).

4-β-Diethylaminoethylamino-6-methoxyquinoline.—4-Chloro-6-methoxyquinoline (2·0 g.), β-diethylaminoethylamine (3·5 c.c.), and potassium iodide (0·01 g.) were heated at 160—170° for 6 hours. The mixture was dissolved in 10% acetic acid (20 c.c.) and sodium acetate (1·0 g.), treated with charcoal, made alkaline, and extracted with benzene. After removal of the benzene from the dried extract, the *product* was distilled in a vacuum and crystallised from light petroleum, m. p. 99° (Found: C, 69·8; H, 8·35; N, 14·9. $C_{16}H_{23}ON_3$ requires C, 70·3; H, 8·45; N, 15·4%).

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Research Laboratories, Blackley, Manchester, 9. [*Received, October 16th*, 1948.]

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312. *A Comparison of the Dipole Moments of the Isomeric Benzaldoximes and Furfuraldoximes.*

By K. E. Calderbank and R. J. W. Le Fèvre.

The dipole moments of monomeric oximes may be calculated from measurements on dilute solutions. Acetoxime, the benzaldoximes, and the furfuraldoximes are taken as examples. Results are compatible with present-day views of the structural relationships of these isomers. Some qualitative high-frequency conductivity comparisons in water are also recorded which parallel known behaviour in liquid sulphur dioxide. Data for benzaldehyde, *o*-nitrobenzaldehyde, and furfuraldehyde are also included.

Hassel and Naeshagen (*Z. physikal. Chem.*, 1924, *B*, **4**, 217) attempted measurements of the dipole moment of benzaldoxime in benzene solution, but refrained from evaluating their results because of the known association of the solute in this solvent (Beckmann, *Ber.*, 1888, **21**, 767). Later, Sutton and Taylor (*J.*, 1931, 2190; 1933, 63), when selecting compounds with which to provide a physical demonstration of the absolute configuration of isomeric oximes, chose to work with the related *N*-ethers, whose unpolymerised condition was definitely established.

However, an inspection of Beckmann's data indicates that, within the concentration ranges usual for dipole-moment determinations, several oximes are monomeric or nearly so; hence,

an approach similar to that made with the sulphonamides or the fatty acids (Le Fèvre and Vine, *J.*, 1938, 1790, 1795) should easily reveal the polarity of the single molecules.

Appropriate measurements have therefore been made with the substances named in the title, together with acetoxime, in benzene or chloroform (see Experimental). Evidence of dissociation with increasing dilution is seen by a non-rectilinearity of either dielectric constant or density with composition. When the formulæ $\varepsilon_{12} = \varepsilon_2(1 + \alpha w_1)$ and $d_{12} = d_2(1 + \beta w_1)$ are used (see *J.*, 1937, 1805, for explanation), $\alpha\varepsilon_2$ and βd_2 are not constant but smoothly related to w_1, so that values of α and β for $w_1 = 0$ are available by extrapolation, and from them the moments of the monomeric oximes, *viz.* (in D.):

	M. p.	In C_6H_6.	In $CHCl_3$.
α-Benzaldoxime	35°	1·2	0·7
β- ,,	128	$1·5_5$	$1·2_5$
α-Furfuraldoxime	75	1·2	$0·8_2$
β- ,,	92	1·9	—
Acetoxime	61	0·9	—

Vectorial analysis is made difficult by possible intramolecular rotation of the angular polar hydroxyl and/or furyl groups. The cases will be discussed separately. To remove ambiguity, we use the prefix *cis-* or *trans-* with respect to the aldoximino- H and HO groups.

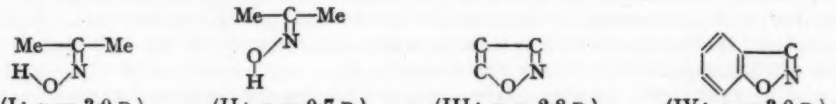
Benzaldoximes.—Maximum and minimum resultant moments will occur when the O–H bond lies in the plane containing the C=N and N–O components. They have been calculated from the following link moments and bond angles: H–C, 0·4; N–O, 0·3; C=N, 2·0; H–O, 1·6; Ph–C–H, 110°; C=N–O, 121°; N–O–H, 105°. The last two are, respectively, the N=N–Ph angle in *trans*-azobenzene (Robertson and Woodward, *Proc. Roy. Soc.*, 1939, *A*, **171**, 398) and the H–O–H angle in water (Darling and Dennison, *Physical Rev.*, 1940, **57**, 128). The N–O link moment has been deduced from Sutton and Taylor's second paper (see above). The phenyl nucleus is assumed to have a regular hexagonal form. Accordingly, we estimate that the *trans*-oxime may have $\mu = 3·6$—$1·4$ D., and its *cis*-counterpart $\mu = 3·7$—$1·0$ D.

The moments as measured are seen to be of the order of the respective minima. The implication is therefore that the hydroxyl group is not freely rotating. In one configuration, at least, this may be ascribed to steric hindrance, in both perhaps to the mutual potential energy–azimuthal angle relation between the two larger moments, *viz.*, $\mu_{C=N}$ and μ_{HO}.

As to the former cause, the annexed figures *a*, *b*, *c*, and *d* correspond to the extreme structures. They show the sections which the constituent atoms would make in the plane of the paper during "free rotation." Being drawn from the data of Pauling and Huggins (*Proc. Nat. Acad. Sci.*, 1932, **18**, 293), they can be regarded as minimum estimates. Superimposed on one version of the *trans*-form is the dotted outline of the "Wirkungs-radien" of the atoms immediately concerned (Stuart, *Z. physikal. Chem.*, 1935, *B*, **27**, 353). A measure of interference with the "free rotation" of the hydroxyl group can therefore be predicted for this isomeride. As a result, its hydrogen atom is likely to inhabit regions more remote from the phenyl. In addition to this cause, however, it is possible that the O–H disposition is strongly affected in all oximes by dipolar repulsions. For a rough approximation we have considered only $\mu_{C=N}$ and μ_{HO}. Using the various dimensions given above in conjunction with enlarged drawings of the two limiting hydroxyl positions in the C=N–OH unit, and making appropriate substitutions in the equation for the potential energy due to the mutual arrangement of two dipoles [*i.e.*, $U = (\cos\chi - 3\cos\alpha_1\cos\alpha_2)\mu_1\mu_2/r^3$; cf. Jeans, "Electricity and Magnetism," Cambridge University Press, 1920, 354], the energy difference calculable is $0·36 \times 10^{-12}$ c.g.s unit (*i.e.*, *ca.* $8kT$ at ordinary temperatures) in favour of hydroxyl dispositions such as in *b* and *d*. We therefore suggest that configuration allotment should be made on the basis of the estimated least values, *i.e.*, that in the oximes, m. p. 35° and 128°, the H and HO groups are *cis-* and *trans-*, respectively.

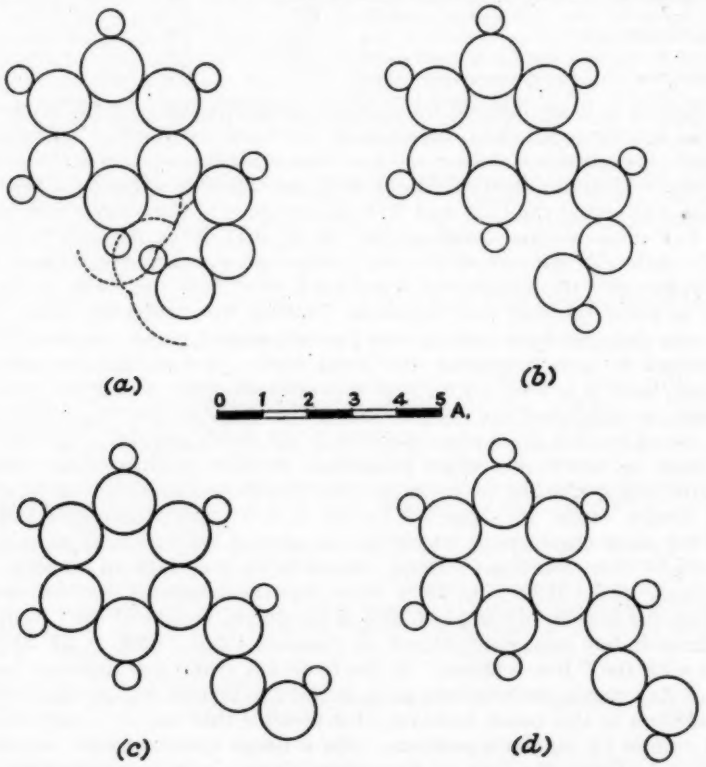
Furfuraldoximes.—*A priori* calculation of resultants requires the resolution of one fixed vector with two others, each rotating around its own axes. A general solution for this type of problem appears to be cumbersome; that of Fuchs (*Z. physikal. Chem.*, 1931, *B*, **14**, 339) is a special case. We have therefore again had recourse to scale drawings; taking $\mu = 0·7$ D. (Smyth and Walls, *J. Amer. Chem. Soc.*, 1932, **54**, 3230) symmetrically through the oxygen atom as the appropriate vector for the furan nucleus (regarded as a regular pentagon), and other values as above, maxima and minima are calculable as 3·7 and 0·3 D. for the *cis-* and 3·4 and 1·3 for the *trans*-furfuraldoxime, respectively. By parallel arguments to those used above, the observed moments should follow the minima expected, *i.e.*, the oxime, m. p. 75°, is the *cis*-compound, and that of m. p. 92° the *trans-*.

Acetoxime.—Comparison of the moment found (*ca.* 1 D.) with those expected on the basis of the values used above again suggests that the configuration conforms much more to (II) than to (I).



(I; μ = 3·0 D.) (II; μ = 0·7 D.) (III; μ = 2·8 D.) (IV; μ = 3·0 D.)

General support for our discussion is also seen in the moments reported for *iso*oxazole (III) and indoxazen (IV) by Jensen and Friediger (*Kgl. Danske Videnskab. Selskab.*, 1943, **20**, No. 20), since it is reasonable (μ_{C-O} being less than μ_{H-O}) that the polarity of (III) (2·8) should approach



but fall slightly short of that of (I) (3·0, estimated), and similarly (IV) (3·0) that of Fig. *a* (3·6, estimated). Hassel and Naeshagen (*loc. cit.*) found the moment of the *O*-methyl ether of α-benzaldoxime to be *ca.* 0·9 D., a fact which likewise falls into line with our present conclusions.

Further, it is helpful to note that the infra-red spectrum of acetoxime in carbon tetrachloride (Buswell, Rodebush, and Roy, *J. Amer. Chem. Soc.*, 1938, **60**, 2239, 2444, 2528) displays no indication of the N–H frequency (as would be the case if an appreciable quantity of nitrone form were present), but instead gives clear signs of normal hydroxyl absorption at 2·76 μ together with " associated " hydroxyl absorption at *ca.* 3 μ. As the concentration increases, the latter peak becomes relatively more pronounced (as with alcohols; Buswell, Deitz, and Rodebush, *J. Chem. Physics*, 1937, **5**, 501; Fox and Martin, *Proc. Roy. Soc.*, 1937, *A*, **162**, 419). The 3 μ band is still slightly visible in 0·001M-solutions. The Raman spectrum of acetoxime shows the characteristic hydroxyl displacement (Bernstein and Martin, *Trans. Roy. Soc. Canada*, 1937, **31**, III, 95, 105).

Evidence from Other Sources.—The conclusions regarding the benzaldoximes and furfuraldoximes are in agreement with those reached *via* chemical considerations by Brady, Meisenheimer, Beckmann, and others (see *Ann. Reports*, 1925, **22**, 105 for summary and references).

Physical evidence has not been entirely unequivocal. Brady and Goldstein (*J.*, 1926, 1918) commented that the dissociation constants (deduced indirectly through the sodium salts) of the two benzaldoximes would be in discordance with Ostwald's experience with olefinic acids (*Z. physikal. Chem.*, 1892, **9**, 553) *if*, in the form m. p. 35°, the phenyl and the hydroxyl were not vicinal. The difficulty has since been cleared by conductivity observations directly on the two oximes themselves in liquid sulphur dioxide by Patwardhan and Deshapande (*J. Indian Chem. Soc.*, 1947, **21**, 135) who, however, examined only one furfuraldoxime (that of m. p. 92°).

We have made several comparisons of the benz- and furfur-aldoximes in aqueous solutions of high dilution using the high-frequency conductivity apparatus described by Blake (*Chem. and Ind.*, 1946, 28; *J. Sci. Instr.*, 1945, **22**, 174; 1947, **24**, 77, 101). The most conductive was the furfuraldoxime, m. p. 92°, followed in turn, by its isomer of m. p. 75°, then by the benzaldoxime of m. p. 128°, and lastly by that of m. p. 35°. We infer, therefore, that the oximes of m. p. 92° and 128° have the *trans*-hydrogen–hydroxyl arrangement.

Finally, we note that the refractometric data, due to Auwers and Ottens (*Ber.*, 1924, **57**, 446), for certain aldoximes and their *O*-ethers, also support configurations which are the inversion of those originally written by Hantzsch (*Ber.*, 1891, **24**, 13). It follows from Silberstein's treatment (*Phil. Mag.*, 1917, **33**, 92, 521) that of two isomers that which is more elongated will have the greater molecular refraction. Thus the forms of R·CH:N·OH in which the H and OH are *cis* (*i.e.*, R and HO, *trans*-) should be distinguishable by larger values of $[R_L]$. The following examples are from Auwers and Ottens's paper (*loc. cit.*) and refer to determinations in quinoline :

	M. p.	$[R_L]_D$.		M. p.	$[R_L]_D$.
Benzaldoximes	35°	$36{\cdot}8_3$	Furfuraldoximes	73—74°	29·85
	128	36·5—36·6		89	29·4

In each case therefore the lower-melting form appears to contain a *cis*-disposition of H and OH. A similar conclusion, but based upon ultra-violet absorption spectra, has recently been reached by Raffauf (*J. Amer. Chem. Soc.*, 1946, **68**, 1765) for the furfuraldoximes.

Experimental.

Materials.—The benzaldoximes were prepared by Beckmann's method (*Ber.*, 1890, **23**, 1684), followed by three recrystallisations, effected by raising benzene solutions of the α-oxime or ether solutions of the β-oxime to saturation point at room temperature by adding light petroleum, and cooling. The directions of Brady and Goldstein (*J.*, 1927, 1960) were used for the furfuraldoximes. The α-form was thrice recrystallised from light petroleum–benzene, and the β-form similarly from 10% ethyl alcohol, m. p.s being then 75—76° and 92—93°, respectively. The aldehydes were distilled, immediately before use, in an atmosphere of nitrogen. The *o*-nitrobenzaldehyde used (from *o*-nitrotoluene, see *Org. Synth.*, **24**, 95) had m. p. 87—88°, and the acetoxime, m. p. 60—61°.

Observations.—The apparatus, technique, and symbols used have been explained previously (Le Fèvre and Calderbank, *J.*, 1948, 1949; Le Fèvre and Northcott, *J.*, 1948, 333). In Table I, the total polarisations of the solutes are evaluated for each concentration by Sugden's mixture rule (*Trans. Faraday Soc.*, 1934, **30**, 720) to display the manner of their dependence upon w_1.

Results.—In Table II we show *inter alia* the values of $a\varepsilon_2$ and β at infinite dilution, extrapolated from the preceding data; the calculations otherwise follow our normal routine (see *J.*, 1937, 1805; or "Dipole Moments," Methuen, 2nd edn., 1948, 41).

Previous or Related Measurements.—Coomber and Partington (*J.*, 1938, 1444), in recording the moment of benzaldehyde as 2·96 D., commented upon the disagreement between their value and those of Williams (2·75; *J. Amer. Chem. Soc.*, 1928, **50**, 2350) and Goebel and Wenzke (2·77; *ibid.*, 1937, **59**, 2301). Our redetermination ($2{\cdot}9_8$) thus supports the work of the first-named authors. For furfuraldehyde, Hassel and Naeshagen (*Z. physikal. Chem.*, 1930, *B*, **6**, 441) reported 3·57 D. (against $3{\cdot}6_3$ D. now found by us) at an unstated temperature; however, their orientation polarisation (270·7 c.c.) is not greatly less than ours (272·4 c.c.) and is shown as P_{A+O}. The differences in final μ may therefore come from the ways in which allowances have been made for P_A.

Dobroserdow (see "Tables annuelles," 1913, **2**, 350; Russian original not available) in 1911 measured the dielectric constants (at $\lambda = 60$ cm.) of the four pure oximes listed in the following table, and Walden later (*Z. physikal. Chem.*, 1910, **70**, 580) repeated the first at $\lambda = \infty$. Combination of their figures with the densities and refractive indices given by Trapesonzjanz (*Ber.*, 1893, **26**, 1433) and Brühl (*Z. physikal. Chem.*, 1895, **16**, 216) enables the respective orientation polarisations to be calculated by Onsager's formula $_OP = M(\varepsilon - n^2)(2\varepsilon + n^2)/\varepsilon d(n^2 + 2)^2$ (*J. Amer. Chem. Soc.*, 1936, **58**, 1486) :

	t.	ε_{20}.	d_4^{20}.	n_D^{20}.	$(_OP)_{20}$.	$\mu_{Onsager}$.
Acetaldoxime (Dobr.)	20·5°	3·52	0·9656	2·03	14·5	$0{\cdot}8_2$
,, (Walden).....	22·6	2·98	0·9590	2·02	9·8	$0{\cdot}6_9$
Benzaldoxime	20·1	3·55 *	1·1111	2·50	15·3	$0{\cdot}8_5$
Methyl ethyl ketoxime	19·8	3·4	0·9232	2·08	19·5	$0{\cdot}9_6$
Methyl propyl ketoxime	19·8	3·27	0·9095	2·09	20·7	$0{\cdot}9_9$

* Dobroserdow's value coincides with the mean of earlier determinations by Löwe and Drude, listed in Landolt–Börnstein's "Tabellen," 1912 edn., p. 1219.

TABLE I.

10^6w_1.	$\varepsilon^{25°}$.	$a\varepsilon_2$.	$d_4^{25°}$.	βd_2.	${}_TP_1$.
(a) *Solvent:* Benzene.					
(i) *Benzaldehyde.*					
0	2·2725	—	0·87378	—	—
5,057	2·3194	9·27	0·87456	0·154	212·6
10,166	2,3696	9·55	0·87537	0·156	215·7
14,883	2·4121	9·38	0·87597	0·147	210·9
20,985	2·4681	9·32	0·87686	0·147	207·4
24,932	2·4977	9·03	0·87736	0·144	200·8
(ii) o-*Nitrobenzaldehyde.*					
3,217	2·3160	13·5	0·87480	0·317	412·7
17,513	2·5132	13·7	0·87906	0·301	401·4
(iii) *Benzaldoxime*, m. p. 35°.					
0	2·2725	—	0·87165	—	—
1,891	2·2755	1·59	0·87210	0·238	58·7
4,047	2·2774	1·21	0·87255	0·222	58·4
5,738	2,2794	1·20	0·87279	0·199	62·0
7,191	2·2810	1·18	0·87303	0·192	61·2
(iv) *Benzaldoxime*, m. p. 128°.					
1,555	2·2754	1·87	0·87189	0·154	76·4
1,989	2·2761	1·81	0·87192	0·136	76·0
3,724	2·2777	1·40	0·87227	0·166	65·4
(v) *Furfuraldehyde.*					
0	2·2725	—	0·87145	—	—
3,128	2·3225	16·0	0·87258	0·361	304·1
5,359	2·3542	15·3	0·87306	0·282	291·4
8,157	2·3960	15·2	0·87370	0·276	288·0
9,824	2·4177	14·8	0·87402	0·262	280·9
11,733	2·4485	15·0	0·87456	0·265	282·7
(vi) *Furfuraldoxime*, m. p. 75—76°.					
1,812	2·2749	1·32	0·87180	0·193	56·3
2,413	2·2754	1·20	0·87182	0·153	55·9
4,160	2·2777	1·25	0·87224	0·190	55·6
6,152	2·2800	1·22	0·87280	0·219	53·8
8,978	2·2832	1·19	0·87346	0·222	53·0
(vii) *Furfuraldoxime*, m. p. 92—93°.					
17,70	2·2800	4·24	0·87214	0·390	120·0
3,295	2·2859	4·07	0·87279	0·407	140·6
4,011	—	—	0·87308	0·406	—
5,288	2,2883	2·99	0·87333	0·356	83·3
7,895	2·2928	2·57	0·87400	0·323	77·2
11,408	2·2968	2·13	0·87485	0·298	69·2
16,939	2·3041	1·87	0·87590	0·263	65·1
(viii) *Acetoxime.*					
0	2·2725	—	0·87378	—	—
2,154	2·2753	1·30	0·87385	0·033	41·8
3,469	2·2767	1·21	0·87385	0·020	40·8
5,221	2·2786	1·17	0·87399	0·040	39·7
8,985	2,2810	0·95	0·87417	0·043	36·6
17,026	2·2862	0·81	0·87454	0·045	34·6
(b) *Solvent:* Chloroform.					
0	4·7240	—	1·4678	—	—
(i) *Benzaldoxime*, m. p. 35°.					
1,442	4·7216	−1·66	1·4674	−0·277	44·7
2,392	4·7182	−2·42	1·4668	−0·418	45·1
4,024	4,7133	−2·66	1·4661	−0·422	44·5
6,359	4·7029	−3·32	1·4646	−0·500	41·9
(ii) *Benzaldoxime, m. p.* 128°.					
1,910	4·7195	−2·36	1·4664	−0·733	55·8
2,470	4·7166	−3·00	1·4660	−0·729	47·7
3,606	4·7113	−3·52	1·4655	−0·638	46·3
4,923	4·7000	−4·88	1·4635	−0·873	46·1
(iii) *Furfuraldoxime*, m. p. 75—76°.					
2,114	4·7191	−2·32	1·4672	−0·284	37·7
3,776	4·7156	−1·99	1·4665	−0·344	41·6
6,861	4·7108	−1·92	1·4660	−0·262	39·9

TABLE II.

Substance and mol. wt.	Data source.		p_2.	C.	$a\varepsilon_2$.	β.	${}_\infty P_1$.	$[R_L]_D$.	μ, D.
Benzaldehyde (106·1)	*a*	(i)	0·3409	0·1881	9·31	0·171	215·8	32·0	$2·9_8$
o-Nitrobenzaldehyde (151·1)	*a*	(ii)	0·3409	0·1881	13·6	0·354	420·4	37·3	$4·3_9$
Benzaldoxime, m. p. 35° (121·1)	*a*	(iii)	0·3417	0·1885	1·65	0·294	68·1	36·8 *	$1·2_8$
	b	(i)	0·3773	0·0452	−1·43	−0·183	46·2	36·8 *	$0·6_6$
Ditto, m. p. 128° (121·1)	*a*	(iv)	0·3417	0·1885	2·23	0·150	86·1	36·6 *	$1·5_5$
	b	(ii)	0·3773	0·0452	−0·879	−0·619	69·2	36·6 *	$1·2_5$
Furfuraldehyde (96·1)	*a*	(v)	0·3418	0·1886	15·2	0·332	297·8	25·4	$3·6_8$
Furfuraldoxime, m. p. 75° (111·0)	*a*	(vi)	0·3418	0·1886	1·29	0·184	58·0	$29·8_5$ *	$1·1_7$
	b	(iii)	0·3773	0·0452	−2·40	−0·328	43·6	$29·8_5$ *	$0·8_2$
Ditto, m. p. 92° (111·0)	*a*	(vii)	0·3418	0·1886	4·23	0·558	105·3	29·4 *	$1·9_8$
Acetoxime (73·1)	*a*	(viii)	0·3409	0·1881	1·33	0·032	40·9	24·0	$0·9_6$

* Data from Auwers and Ottens, *loc. cit.*

The moments so estimated for the two ketoximes are only slightly higher than that now found for acetoxime in dilute benzene solution, and accordingly we infer that for them also type (II) is a preferred configuration. The same is probably true for the one known form of acetaldoxime (Beilstein, "Handbuch," **1**, 608). For benzaldoxime, μ_{Orange} appears to be definitely less than that determined in benzene. This disparity could be either a dispersion or a solvent effect. We suspect the latter since a somewhat parallel diminution of apparent moment occurs when benzene is replaced by chloroform (compare corresponding *a* and *b* data in Tables I and II). No exact regularity should, however, be expected, since the so-called "solvent effects" in dipole-moment measurements often include contributions which are solute-dependent (cf. Le Fèvre, *op. cit.*, Chap. III). The following data for five assorted solutes illustrate the point:

Solute.	Apparent μ in C_6H_6.	Apparent μ in $CHCl_3$.	Ratio.
C_6H_5Cl *	1·59	1·18	1·35
$C_6H_5NO_2$ *	4·05	3·05	1·33
$CHCl_3$ *	1·22	1·10	1·11
Paraldehyde †	2·03	2·26	0·90
Benzil ‡	3·76	3·21	1·17

* Le Fèvre and Russell, *J.*, 1936, 491.
† Le Fèvre and Russell, *J.*, 1936, 496.
‡ Caldwell and Le Fèvre, *J.*, 1939, 1614.

Our results (Table II) show a similarly inconstant "solvent" influence:

Benzaldoxime, m. p. 35°	1·23	0·68	1·81
,, m. p. 128°	1·55	1·25	1·24
Furfuraldoxime, m. p. 75°	1·17	0·82	1·43

Equilibrium between the Furfuraldoximes.—Patterson, Buchanan, and Summers (*J.*, 1941, 606) noted that, although these oximes are not interconverted at 20° in ethyl tartrate, yet at 70° an equilibrium is eventually produced. Polarimetrically this appeared to contain 45% and 55%, respectively, of the higher- and the lower-melting form.

To obtain a preliminary indication of the rôle of the solvent in such a process, we kept pure specimens of the two isomers at 100° for 14 hours and then made routine polarisation measurements in benzene:

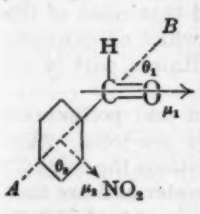
10^6w_1.	$\varepsilon^{25°}$.	$a\varepsilon_2$.	$d_4^{25°}$.	βd_2.	P_1.
0	2·2725	—	0·87145	—	—
	Preparation from form, m. p. 92—93°.				
2361	2·2763	1·61	0·87196	0·216	61·4
	Preparation from form, m. p. 75—76°.				
3445	2·2780	1·60	0·87236	0·264	59·4

From our previous data (Tables I and II) the total polarisations of the pure oximes at these concentrations are seen to be 105 and 56 c.c. Application of the simple mixture formula to the corresponding orientation polarisations, therefore, shows contents of the higher- and the lower-melting oximes in the ratios *ca.* 7 : 93 and 11 : 89 in the two cases. The inference is, therefore, that the equilibration has a marked solvent dependence.

o-Nitrobenzaldehyde.—The moment now recorded for this substance completes the extant data for the three nitrobenzaldehydes:

o-, μ = 4·30 D. (present work).
m-, 3·2$_8$ D. (Hassel and Naeshagen, *Chem. Zentr.*, 1930, 1956).
p-, 2·4 D. (Williams, *J. Amer. Chem. Soc.*, 1928, **50**, 2350).
2·41 D. (Coomber and Partington, *loc. cit.*).

At the outset we expected a higher polarity for the *o*-compound since $\mu_{C_6H_5\cdot NO_2}$ exceeds that of $\mu_{C_6H_5\cdot CHO}$, and $\mu_{o\text{-phthalaldehyde}}$ is known to be 4·5 D. (Hassel and Naeshagen, *loc. cit.*). Coomber and Partington (*loc. cit.*) had concluded that, because of induced and mesomeric moments in the aromatic ring, the resultant moment, μ_1, of the benzaldehyde molecule is oriented effectively at 143½° to the 1 : 4-axis, *AB*. In the annexed diagram we put $\mu_1 = 2{\cdot}9_8$, $\mu_2 = 4{\cdot}03$, $\theta_1 = 36\frac{1}{2}°$, and $\theta_2 = 120°$, 60°, and 0°, respectively, for the *o*-, *m*-, and *p*-structures, and consider collinear and normal components to *AB* during rotation around *AB* of the planes containing the aldehydo- and the nitrophenyl radicals. Taking χ as the azimuthal angle thus involved, and noting that at all values of χ the components normal to *AB* must produce a resultant which is also normal, we have for the square of the molecular resultant of a given configuration:



$$R^2 = (\mu_1 \cos\theta_1 - \mu_2 \cos\theta_2)^2 + \mu_1^2 \sin^2\theta_1 + \mu_2^2 \sin^2\theta_2 + 2^2\mu_1\mu_2 \sin\theta_1 \sin\theta_2 \cos\chi.$$

"Free rotation" or $\chi = 90°$ both require a solution in which the last term is zero. Our calculations may be summarised:

	$\chi = 0°$.	$\chi = 180°$.	"Free rotn."	Obs.
o-$NO_2\cdot C_6H_4\cdot CHO$	6·9	4·7	5·9	4·3
m- ,,	5·3	1·8	3·9	3·3
p- ,,	2·4	2·4	2·4	2·4

Although we admit that assumptions which are satisfactory for *m*- or *p*-disubstituted benzenes are uncertain when applied to *o*-isomers (cf., *e.g.*, Smallwood and Herzfeld, *J. Amer. Chem. Soc.*, 1930, **52**, 1919), we submit that the low moment of *o*-nitrobenzaldehyde now found indicates that its molecule exists extensively as configurations in which χ is *ca.* 180°. As such, the aldehydic hydrogen is correctly disposed for the easy photochemical substitution by oxygen (Zimmer, *Z. physikal. Chem.*, 1933, *B*, **23**, 239; Leighton and Lucy, *J. Chem. Physics*, 1934, **2**, 756) and eventual formation of *o*-nitrosobenzoic acid.

The possibility of following the last change, under controlled conditions of illumination and temperature, *via* dielectric-constant readings, has also been explored, but unsuccessfully owing to the extremely low solubility of the nitroso-acid in all non-conducting solvents tried.

Certain of these measurements were initiated at University College, London, during 1938—9 in collaboration with Mr. C. J. Rayner and as the result of discussions with Dr. O. L. Brady. We are indebted to the latter for gifts of the isomeric benzaldoximes, to the Chemical Society for apparatus, and to the Commonwealth Science Fund for financial assistance.

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313. *The Polysaccharides of the Florideæ. Floridean Starch.*

By V. C. BARRY, T. G. HALSALL, E. L. HIRST, and J. K. N. JONES.

The polysaccharide designated Floridean starch, which is known to occur in many red algæ, has been isolated from the fronds of *Dilsea edulis*. It is shown to consist entirely of glucose residues. Investigation of its oxidation by the periodate ion shows that it is structurally different from normal starches or glycogens in that it contains a large proportion of 1 : 3-linked glucose residues. It is resistant to attack by crystalline β-amylase.

GRANULAR material which gives a colour with iodine has been observed in various red algæ when sections of the latter are examined under the microscope [see, *e.g.*, Nägeli, "Die Stärkekorner," 1865, p. 533; Belzung, *Ann. Sci. Nat.*, (*a*) *Botanique*, 1887, (vii), **5**, 179; Kylin, *Z. physiol. Chem.*, 1913, **83**, 174; Colin, *Compt. rend.*, 1933, **197**, 423]. The colour with iodine is not the characteristic blue shown by normal starch, but varies from deep violet to brown. It was attributed to a polysaccharide designated Floridean starch which was said to have certain properties in common with starch and glycogen. For instance, hydrolysis of the polysaccharide with amylolytic enzymes resulted in the formation of degraded products which gave no colour with iodine. These observations were, however, not made on purified material, and the so-called Floridean starch was not actually isolated until Colin (*loc. cit.*) obtained from *Lemanea*, a red fresh-water alga, a glycogen-like substance which had $[\alpha]_D$ +105°, gave a violet colour with iodine, and yielded D-glucose on hydrolysis. This author demonstrated the presence, in some twenty red algæ, of a substance which gives a coloration with iodine. The brown algæ, on the other hand, contain laminarin as a reserve polysaccharide and are devoid of Floridean starch, whilst the green algæ, for example *Ulva lactuca*, contain particles which give a colour with iodine and potassium iodide very similar to that shown by starch.

The difficulties encountered in the isolation and purification of Floridean starch are not unexpected, since aqueous extracts of marine red algæ always contain a complex mixture of polysaccharides. It is possible, therefore, that this Floridean starch may be present also in other red algæ, thereby adding still another complication to structural studies in this group. *Chondrus crispus*, for example, undoubtedly contains some Floridean starch, and, in interpreting the results of experiments with carragheen mucilage, it must be ascertained that none of this material is present adventitiously before it can be concluded that the glucose which accompanies the galactose in the hydrolysis products of the mucilage does in fact constitute a part of the mucilage molecule.

In the present paper an account is given of the isolation, purification and preliminary examination of Floridean starch from *Dilsea edulis*. The fronds of this sea-weed, when extracted with cold, dilute hydrochloric acid, yield a viscid solution which contains the sulphate ester of a galactan. The disintegrated fronds are washed with cold distilled water and are then suspended in boiling water for about an hour. The filtered aqueous extract is poured into an excess of alcohol or acetone, giving a white colloidal precipitate which, after further purification, is finally obtained in the form of a cream-coloured powder. This swells in cold water and then dissolves giving an opalescent solution which displays a faint reducing power towards Fehling's solution. It gives with iodine in aqueous potassium iodide a colour varying from brownish-red to deep violet according to the concentration of the reagents. This colour disappears on heating and reappears on cooling. As ordinarily obtained, the product contains some 10% of ash which can, however, be much reduced by dialysis. The ash contains phosphate, and, apparently, combined phosphorus occurs in the molecule as it does in certain starches. The polysaccharide resembles glycogen in that it is not precipitated by half-saturation with ammonium sulphate, but is so by full saturation. It is composed entirely of glucose residues and gives on hydrolysis with mineral acid a yield of 96% of glucose, determined polarimetrically.

No sugar other than glucose can be detected when the products of hydrolysis are examined on the paper chromatogram using the method of Partridge (*Nature*, 1946, **158**, 270; *Biochem. J.*, 1948, **42**, 238). It has been claimed (Kylin, *Kgl. Fysiograf. Sällskap. Lund. Förh.*, 1943, **113**, 51) that Floridean starch gives maltose on hydrolysis with dialysed malt extract. This would point to the occurrence of maltose residues in the polysaccharide, but no crystalline derivative of the sugar has yet been reported from these hydrolyses. In the present paper it is shown that the yield of maltose obtainable by treatment with a solution of crystalline β-amylase is, at best, very small, an apparent conversion into maltose of no more than 3% being observed. This was not due to poisoning of the enzyme since normal hydrolysis of waxy-maize starch occurred in the presence of the Floridean polysaccharide.

Oxidation of the polysaccharide with periodic acid results in a pronounced fall in the specific rotation. The final value ($[\alpha]_D$ +10°) is very close to that reported for oxidised maize starch (+8°) by Jackson and Hudson (*J. Amer. Chem. Soc.*, 1937, **59**, 2049; 1938, **60**, 989). In the course of this oxidation the polysaccharide consumes 0·6 mol. of periodate per glucose residue, 40% of the glucose residues remaining unattacked. It is probable, therefore, that some of the glucose residues are not linked through C_1 and C_4 as in amylose, but through C_1 and C_3 as in laminarin. The yield of formic acid after oxidation with potassium periodate corresponds to the production of 1 mol. of acid per 18 glucose residues, but, since the polysaccharide displayed some reducing power, it is possible that much of the formic acid came from reducing terminal groups, and no precise conclusions can be drawn at present regarding the presence or absence of branched chains in the Floridean polysaccharide. It is apparent, however, that the substance differs markedly in structure from starch and glycogen in that it contains, in addition to 1 : 4-linked glucose residues, other glucose units mutually linked through the 1 : 3-positions. In this connection it is of interest to compare the properties of the Floridean polysaccharide with those of lichenin which has been recently investigated by Meyer and Gürtler (*Helv. Chim. Acta*, 1947, **30**, 751; see also Boissonnas, *ibid.*, p. 1703), and it is possible that the Floridean polysaccharide bears a relation to starch similar in type to that which lichenin does to cellulose. The high specific rotation of Floridean starch points to the presence of α-glucosidic links, but the maximum value so far recorded ($[\alpha]_D$ +156° in water) is so much lower than the corresponding figures for starch and glycogen (*ca.* 200°) that it cannot be regarded as certain that all the links are of the α-type, even when possible differences in molecular structure are taken into account. In this connection it is significant that the acetate obtained from Floridean polysaccharide has a rotation much lower than that of starch acetate.

Experimental.

Isolation of Floridean Starch.—Sections of the fronds of *Dilsea edulis*, when treated with iodine in aqueous potassium iodide, show, under the microscope, granules stained reddish-brown. It has not been possible to devise a method for the isolation of these granules, but the material which is stained reddish-brown was obtained in an amorphous condition by the following procedure. The fronds were first extracted with cold dilute hydrochloric acid as described by Barry (*Proc. Roy. Irish Acad.*, 1945, **50**, *B*, 349) for the removal of a galactan sulphuric ester. The disintegrated fronds were washed exhaustively with distilled water by decantation, until the washings were free from sulphate. They were then suspended in boiling water for 1 hour. After filtration of the hot solution through cloth, it was poured into an excess of alcohol (or acetone), giving a finely-divided white precipitate. This was purified by dissolution in water, clarification on the centrifuge, and reprecipitation by alcohol. Alternatively, purification can be effected by dialysis in dilute hydrochloric acid against distilled water, until all chloride has been removed. If the fronds are treated with boiling water before the extraction with cold aqueous acid has been carried out, no Floridean starch is obtained, the action of the acid on the frond cells being apparently essential in rendering the polysaccharide extractable. It was observed on occasions that even strong aqueous solutions fail to give a precipitate with alcohol until a trace of electrolyte (*e.g.*, calcium chloride) has been added (compare glycogen). The product so obtained appears, after drying, as a cream-coloured powder which shows a very slight reducing action towards Fehling's solution. With iodine and aqueous potassium iodide it gave a colour varying from reddish-brown to violet according to the concentration of the reagents. The product had $[\alpha]_D^{18°}$ +156° in water (*c*, 0·6) [Found : (*a*) reducing power by the hypoiodite method; 5800 g. were equivalent to 1 mol. of hypoiodite; (*b*) N, 0·9%; (*c*) ash, as sulphate, 0·8%). The yield of Floridean starch from 200 g. of air-dried fronds of *Dilsea edulis*, gathered in the early autumn, was 5 g. There may be seasonal variations in the content, but this aspect of the problem has not been studied.

The acetate of Floridean starch was prepared by the method described for starch by Haworth, Hirst, and Webb (*J.*, 1929, 2479). It was obtained by pouring the reaction mixture into water and, after being washed and dried in a vacuum desiccator, it appeared as a heavy white powder resembling glycogen acetate and was insoluble in water and alcohol, but soluble in chloroform and acetone. It was ash free and had $[\alpha]_D^{18°}$ +107° in chloroform.

Hydrolysis of Floridean Starch.—The polysaccharide (101 mg.) was heated for 20 hours with hydrochloric acid (0·54N.; 10 ml.). Whilst being heated, the solution became brown. After hydrolysis

a small amount of the solution was neutralised with aqueous ammonia. Three drops of the neutralised solution were placed on the paper chromatogram, which was developed with the butanol layer resulting from a mixture of butanol (40%), ethanol (10%), and water (50%). Only glucose was detected in the developed chromatogram. An amylose estimation was carried out on the Floridean polysaccharide by means of the potentiometric iodine-titration method of Bates, Rundle, and French (*J. Amer. Chem. Soc.*, 1943, **65**, 142) as modified by Hudson, Schoch and Wilson (*ibid.*, p. 1380). No iodine was taken up, and no point of inflection occurred in the plot of the e.m.f. against volume of iodine added. The material, therefore, contained no amylose.

Oxidation by Periodate.—Floridean polysaccharide (158·4 mg.) was dissolved in water (40 ml.) containing potassium chloride (5 g.), and sodium metaperiodate (0·27M.; 10 ml.) was added as oxidant. The oxidation, and the determination of the resulting formic acid, were carried out according to the method described for glycogen by Halsall, Hirst, and Jones (*J.*, 1947, 1399). The amount of formic acid produced after 150 hours (2·5 mg.) corresponded to the production of 1 mol. of acid per 18 glucose residues. Floridean starch (89·1 mg.) was oxidised by potassium periodate as above, and after 8 days the uptake of periodate was determined by the arsenite–bicarbonate method. One mole of periodate was taken up by 263 g. of Floridean polysaccharide. Thus, 56% of the glucose residues are oxidised.

Floridean starch (577 mg.) was dissolved in water (20 ml.) containing potassium periodate (1·5 g.). The resulting solution was shaken for 8 days, and then filtered to remove excess of potassium periodate. To the filtrate, ethylene glycol (1 ml.) was added, and then barium formate until no further precipitation of barium iodiate occurred. This precipitate was filtered off. Ethyl alcohol was then added to the filtrate until no further precipitation occurred. The precipitate was allowed to settle, and the supernatant liquid decanted. The solid was isolated and dried (yield, *ca.* 250 mg.). The solid proved to be mainly inorganic matter and, after treatment with N-sulphuric acid at 100°, glucose was found to be absent (as shown by tests by the paper-chromatogram method).

The decanted liquor was dialysed against running water for 4 days. It was then evaporated under reduced pressure to a small volume. A large excess of alcohol was added, whereupon a dark coloured precipitate was formed. This was isolated by centrifuging, washed with alcohol and ether, and dried (yield, *ca.* 15 mg.). This solid (11·5 mg.) was hydrolysed in a sealed tube with N-sulphuric acid (0·4 ml.) by heating it in a water-bath for 6 hours. The tube was then opened and the contents neutralised with barium carbonate. The liquid was centrifuged, and the clear hydrolysate examined on a paper chromatogram. One sugar only was present, the position of which corresponded exactly to that of glucose (glucose, galactose, and mannose were used as reference sugars). It is evident therefore, that intact glucose residues are present in the polysaccharide after oxidation of the latter by periodate.

Treatment of Floridean Starch with a Solution of Crystalline β-Amylase.—Through the kindness of Dr. R. K. Balls of the Enzyme Research Laboratory of the United States Department of Agriculture, we were able to obtain a sample of crystalline β-amylase prepared from the sweet potato. Floridean polysaccharide (518·7 mg.) was dissolved in water (43 ml.), and acetate buffer solution (pH 4·8; 5 ml.) and a solution of crystalline β-amylase (2 ml. of 0·04%) were added. The resulting solution was covered with a thin layer of toluene to maintain aseptic conditions and kept in an incubator maintained at 37°. After 42 hours, the reducing power of a 5-ml. portion of the Floridean solution was determined by the hypoiodite method. After allowing for the initial reducing power of the polysaccharide, the apparent conversion into maltose was 3%. In order to determine whether this low figure was due to poisoning of the enzyme by the polysaccharide, two solutions of waxy-maize starch were prepared. One (*A*) contained 69·2 mg. in water (44·5 ml.), and the other (*B*) 54·4 mg. in water (44·5 ml.) to which had been added the Floridean polysaccharide (31·8 mg.). To each of these were added acetate buffer solution (pH 4·8; 5 ml.) and β-amylase solution (0·04%; 0·5 ml.). The solutions were then covered with a thin layer of toluene and kept in an incubator at 37° for 24 hours. After this time the reducing powers of the two solutions were determined by the hypoiodite method. The reducing power of the solution (*A*) corresponded to a 53% conversion into maltose. After allowing for the initial reducing power of the " Floridean starch " in the case of the solution (*B*), the reducing power corresponded to an apparent conversion of 57% of the waxy-maize starch into maltose provided that no production of reducing substances from the Floridean starch had occurred. This indicates that the hydrolysis of waxy-maize starch in the presence of Floridean polysaccharide proceeded to about the same extent as the hydrolysis of the waxy-maize starch in the absence of Floridean polysaccharide. If the slight increase in reducing power is entirely due to hydrolysis of the Floridean polysaccharide, it indicates an apparent conversion into maltose of 8%.

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314. *Toxic Fluorine Compounds containing the C–F Link. Part VI. ω-Fluorocarboxylic Acids and Derivatives.*

By F. J. Buckle, F. L. M. Pattison, and B. C. Saunders.

The synthesis of hitherto undescribed ω-fluorocarboxylic acids and esters, of the type $F\cdot[CH_2]_n\cdot CO_2R$, is described. It has been established that if n is odd, the compound is toxic and causes fluoroacetate-like symptoms in animals. If n is even no such toxic properties are shown. This remarkable alternation of properties is discussed in the light of the β-oxidation theory of long-chain carboxylic acids. This theory does not, however, account for all the facts observed.

The toxicity is often greatly enhanced if $R = CH_2\cdot CH_2F$ compared with R = Me or Et. This difference is less obvious when the chain is long ($n = 7$) and is negligible with the ω-fluorodecoates ($n = 9$).

Previous investigations in this series of papers (Saunders *et al.*, Parts I to V) have shown that any compound which can give rise to fluoroacetic acid (or the fluoroacetate ion) either by hydrolysis or by oxidation (or both) is toxic. The toxic grouping is thus $F\cdot CH_2\cdot CO$, and any substitution in this radical destroys the toxicity. We had reached this conclusion by May 1943 (McCombie and Saunders, Report No. 5 on Fluoroacetates to Ministry of Supply, May 30, 1943; also Carpenter, Kilby, McCombie, and Saunders, Report to Ministry of Supply, January 8, 1944). American workers later showed (private communication) that esters of β-fluoropropionic acid were non-toxic, whereas esters of γ-fluorobutyric acid were toxic. In 1944 (Saunders, Ministry of Supply Meeting, June 1, 1944, and Report No. 11 on Fluoroacetates to Ministry of Supply, August 8, 1944) we reported the synthesis of *ethyl 5-fluoropentanecarboxylate*, $F\cdot[CH_2]_5\cdot CO_2Et$ (I). This was a stable, colourless, liquid and we showed that it possessed very potent toxic properties of the "fluoroacetate" type. By subcutaneous injection of the propylene glycol solution into mice the L.D. 50 was 4 mg./kg. Methyl fluoroacetate (II) may be taken as a convenient standard (Part I, *J.*, 1948, 1773) and has an L.D. 50 of about 6 mg./kg. for saline solutions, and 15 mg./kg. for propylene glycol solutions.* Therefore ethyl 5-fluoropentanecarboxylate was about 7 times as toxic as methyl fluoroacetate (molecule for molecule).

In Part IV (this vol., p. 916) it was shown that 2-fluoroethyl fluoroacetate was about twice as toxic as methyl fluoroacetate (MFA) by inhalation. By analogy then it seemed that *2-fluoroethyl 5-fluoropentanecarboxylate* (III) might be a compound of exceptionally high toxicity. This proved to be correct, for its L.D. 50 for subcutaneous injection into mice was 2·5 mg./kg., *i.e.*, it is about 11 times as toxic as MFA (per molecule) by this route and in propylene glycol as solvent.

The comparison of the toxicities of compounds I, II, and III by intravenous injection into rabbits also revealed a similar gradation, as shown herewith:

	L.D. 50, mg./kg.
Methyl fluoroacetate (II)	0·25
Ethyl 5-fluoropentanecarboxylate (I)	0·2—0·5
2-Fluoroethyl 5-fluoropentanecarboxylate (III)	0·1—0·2

The very high toxicity of ethyl 5-fluoropentanecarboxylate and its derivatives and the "fluoroacetate-like" symptoms produced seemed to be of particular interest since by a process of β-oxidation in the animal body 5-fluoropentanecarboxylic acid would readily give rise to the toxic fluoroacetic acid. Similar remarks apply to γ-fluorobutyric acid and its derivatives prepared by American workers. The non-toxicity of β-fluoropropionic acid and its derivatives may, on the other hand, be due to the inability of this acid to give the toxic fluoroacetic acid by a process of β-oxidation.

In order to prove that fluorine was responsible for the lethal action in (I) and (II), the intermediate bromo-esters were examined physiologically. Ethyl 5-bromopentanecarboxylate was found to be entirely without toxic action, and the toxicity of the *2-fluoroethyl* ester was of a low order, the L.D. 50 being about 75 mg./kg.

We then set out to determine whether this remarkable alternation in toxic properties could be observed among other ω-fluorocarboxylic acids.

Ethyl δ-fluorovalerate (IV) was found to be completely non-toxic, a subcutaneous injection of 160 mg./kg. killing 0/2 mice, with complete absence of any symptoms of poisoning. Intramuscular injection of 40 mg./kg. into rats similarly produced no symptoms.

* This difference in toxicities when using saline and propylene glycol should be noted when comparing potencies.

In striking contrast to this we showed that *ethyl 7-fluoroheptanecarboxylate* (V) was highly toxic and that the *2-fluoroethyl* ester (VI) was slightly more toxic.

Ethyl 9-fluorononanecarboxylate (VII) was found to be even more toxic than ethyl 5-fluoropentanecarboxylate by injection into rabbits, the L.D. 50 for propylene glycol solution of (VII) being 0·2 mg./kg.* Mice and rats were slightly more resistant, but exhibited convulsions of the general fluoroacetate type. On account of the high boiling point of the material, no inhalation experiments were attempted. *2-Fluoroethyl 9-fluorononanecarboxylate* (VIII) was found to be no more toxic than the corresponding ethyl ester by injection into mice. Each had an L.D. 50 of about 10 mg./kg. This apparent anomaly is discussed later.

Ethyl 10-fluorodecanecarboxylate (IX) when injected into mice caused no deaths at a concentration of 100 mg./kg., and produced no symptoms of any kind. Therefore it was non-toxic. In accordance with expectation *ethyl 11-fluoroundecanecarboxylate* (X) proved to be toxic.

The results obtained for injection into mice of propylene glycol solutions are summarised in the following table.

Value of n in acid $F\cdot[CH_2]_n\cdot CO_2H$.	L.D. 50, mg./kg. (propylene glycol as solvent). Me or Et ester.	2-Fluoroethyl ester.	Value of n in acid $F\cdot[CH_2]_n\cdot CO_2H$.	L.D. 50, mg./kg. (propylene glycol as solvent). Me or Et ester.	2-Fluoroethyl ester.
1	15 (Me)	8·5	5	4 (Et)	2·5
2	Non-toxic (American workers)	—	7	9 (Et)	7
			9	10 (Et)	10
			10	>100 (Et) ∴ Non-toxic	—
3	Toxic (American workers)				
4	>160 (Et) ∴ Non-toxic	—	11	<20 (Et)	—

It is thus apparent that, in this series of ω-fluorocarboxylic esters, if n is odd the compound is highly toxic, whereas if n is even it is non-toxic. This striking alternation in toxicity would seem to provide a useful verification of the theory of β-oxidation of fatty acids in the animal body.

The theory of β-oxidation was first put forward by Knoop (*Beitr. Chem. Physiol. Path.*, 1904, **6**, 150; 1906, **11**, 411) and was based essentially on the following evidence. The ω-phenyl derivatives of the fatty acids containing from one to five carbon atoms were administered to dogs, and the urine was subsequently analysed for the presence of derivatives of these acids. In all cases the final acid produced by breakdown was excreted as its glycine derivative. Those fatty acids containing an odd number of carbon atoms were excreted as hippuric acid, and those with an even number as phenylaceturic acid, $CH_2Ph\cdot CO\cdot NH\cdot CH_2\cdot CO_2H$. These results led Knoop to postulate that fatty acids were oxidised by a route which involved the loss of two carbon atoms at each stage, owing to oxidation occurring at the β-carbon atom. He suggested, but without evidence, that the β-oxidation took place by the following steps:

$$R\cdot CH_2\cdot CH_2\cdot CO_2H \xrightarrow{-H_2} R\cdot CH{:}CH\cdot CO_2H \underset{-H_2}{\overset{+H_2O}{\rightleftharpoons}} R\cdot CO\cdot CH_2\cdot CO_2H \xrightarrow{+H_2O} R\cdot CO_2H + CH_2\cdot CO_2H$$

It will readily be seen in our series of ω-fluorocarboxylic acids, that when n is odd, β-oxidation would yield the toxic fluoroacetic acid, whereas when n is even, the compound will be oxidised only as far as the non-toxic β-fluoropropionic acid. The pharmacological results obtained are in complete accord with this hypothesis, and provide verification, of a kind not hitherto achieved, of the process of β-oxidation in the living animal body. Recently, however, Weinhouse, Medes, and Floyd (*J. Biol. Chem.*, 1944, **153**, 689) have inoculated rat-liver slices with one or two fatty acids containing isotopic carbon, and have obtained some evidence for a process of β-oxidation.

Certain aspects of our results, however, while not invalidating the β-oxidation theory of the ω-fluorocarboxylic acids, do indicate that β-oxidation is not the only factor concerned with the alternation of toxic properties.

Ethyl 9-fluorononanecarboxylate is toxic in accordance with expectation, but the magnitude of the toxicity (L.D. 50 for injection into rabbits 0·2 mg./kg.) is *greater* (molecule for molecule) than that of methyl fluoroacetate (L.D. 50, 0·25 mg./kg.). On the basis of the β-oxidation theory alone, the toxicity of the former ester should be *less* than that of the latter, because of the long chain of CH_2 groups which must be burned away in the body before fluoroacetic acid is

* The ester (VII) would probably be even more toxic in saline solution.

produced. It may be, however, that because of its long chain, the higher ester would have a higher fat : water partition coefficient and therefore would pass more readily than the lower ester through the cell membranes, and there break down giving a higher intracellular concentration of fluoroacetic acid.

Reference has already been made to the fact that 2-fluoroethyl 9-fluorononanecarboxylate was no more toxic than the corresponding ethyl ester by injection into mice. This was contrary to expectation, and was investigated in the following manner. One set of mice was injected with ethyl 9-fluorononanecarboxylate in the usual way; a second set of mice had exactly the same injections of this ester and then, almost simultaneously, injections of fluoroethyl alcohol were made corresponding to the amount which would have been liberated had 2-fluoroethyl 9-fluorononanecarboxylate been injected instead. The mice of the second set therefore contained the same amount of fluorine as if they had been injected with the latter ester. The results are tabulated as follows:

	Wt. of nonanecarboxylate alone, mg./kg.		
	20	8	6
Ethyl 9-fluorononanecarboxylate alone	6/6 killed	4/6 killed	4/6 killed
Ditto + fluoroethyl alcohol	6/6 killed	5/6 killed	1/6 killed

This showed the fluoroethyl alcohol had no very marked effect, and this observation was in line with the fact that the ethyl and the 2-fluoroethyl ester had the same toxicity.

Two points are tentatively put forward to account for this similarity of toxicity: (1) As the homologous series of ω-fluoro-esters is ascended, the proportion of fluoroethyl alcohol obtainable from the 2-fluoroethyl esters must decrease. It is suggested that a point will be reached when the amount of fluoroethyl alcohol derived from the 2-fluoroethyl ester will be too small to make any apparent difference in the toxicity. The L.D. 50 of 2-fluoroethyl 9-fluorononanecarboxylate liberates only about 0·05 mg. of fluoroethyl alcohol in the mouse. This, if injected alone, would have no action. (2) 2-Fluoroethyl 5-fluoropentanecarboxylate was found to be nearly twice as toxic as the corresponding ethyl ester, weight for weight; but if its action were due solely to hydrolysis *in vivo* the toxicity should be the same. Similar remarks apply to 2-fluoroethyl fluoroacetate. It thus seems possible that the molecule may exert some action *per se*, independently of any subsequent degradation. It might further be suggested that if the toxic action of these 2-fluoroethyl esters is indeed dependent primarily upon the molecule as a whole (as distinct from its hydrolysis products) then the action may be related to the terminal fluorine atoms of the molecule. If this is so, there may be an optimum stereochemical distance apart of the fluorine atoms for the maximum action of the molecule in this way. It is significant that the difference in activity between the ethyl and the 2-fluoroethyl esters is greater with the shorter chains. With the fluoroheptanecarboxylates the difference is slight, and it disappears entirely with the nonanecarboxylates.

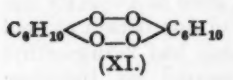
Although our results support the β-oxidation theory, one point must not be overlooked, namely, that fluoroacetic acid is perhaps not the actual toxic agent and has to be converted into some other compound before exerting any activity. American workers (private communication) showed that both methyl γ-fluorobutyrate and methyl γ-fluorocrotonate, $F{\cdot}CH_2{\cdot}CH{:}CH{\cdot}CO_2Me$, were highly toxic, and moreover, we showed that the crotonate was much more rapid in its lethal action than fluoroacetate at equivalent concentrations. However, this is purely hypothetical and is not yet supported by any direct experimental evidence.

Synthetic Methods employed in this Series of Compounds.—As direct chlorination or bromination of a carboxylic acid gives invariably the α-substituted acid, such methods are useless for the preparation of the ω-substituted acids required for the present investigation; *ad hoc* methods have therefore had to be found for the preparation of each individual ω-fluorocarboxylic acid and its derivatives.

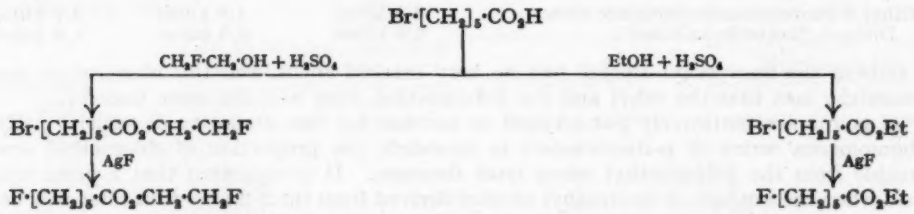
Ethyl δ-fluorovalerate (IV) was prepared in an impure state (67% purity by fluorine analysis) from ethyl δ-bromovalerate, and in a pure condition from ethyl δ-iodovalerate by fluorination with the silver fluoride. It may be noted that difficulty was experienced in converting allylacetic acid into δ-bromovaleric acid. The conditions of the experiment were varied between wide limits both in the presence and the absence of peroxides. Conflicting results had previously been obtained by other workers in this field (Boorman, Linstead, and Rydon, *J.*, 1933, 568, 1974; Kharasch and McNab, *Chem. and Ind.*, 1935, **54**, 98). δ-Iodovaleric acid was prepared by Carter's method (*J. Amer. Chem. Soc.*, 1928, **50**, 1968) who converted it into the ethyl ester using a solution of dry hydrogen chloride in alcohol. We found that under these conditions a large part of the iodo-acid was converted into the chloroester. We therefore carried out the esterification using sulphuric acid and showed that if the

molar ratios of sulphuric acid, iodo-acid, and ethyl alcohol were 1 : 4 : 32, neglible interchange took place. The fluorination of the iodo-ester was achieved by the use of pure dry silver fluoride in the absence of a solvent.

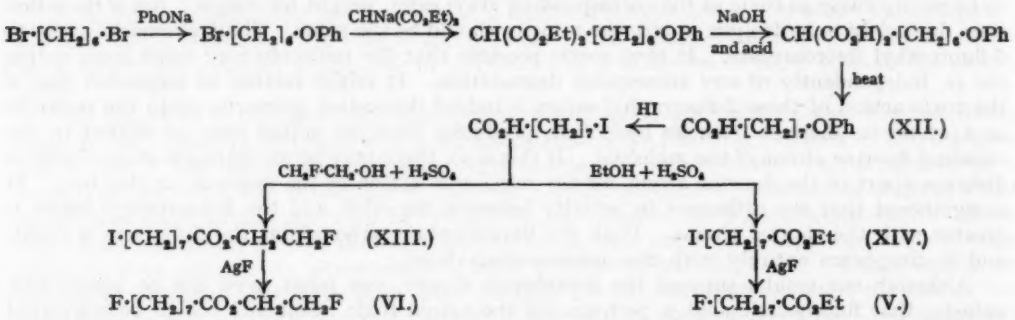
The starting point for the 5-fluoropentanecarboxylic esters was *cyclo*hexanone, which was oxidised to 5-hydroxypentanecarboxylic acid by Robinson and Smith's method (*J.*, 1937, 373). This was then converted into the bromo-acid by means of hydrogen bromide and sulphuric acid (Barger, Robinson, and Smith, *J.*, 1937, 718). By employing certain modifications described in the Experimental section, the yields have been increased. It may be noted that a solid of m. p. 130°, probably di*cyclo*hexylidene peroxide (XI), could usually be isolated during the preparation of 5-hydroxypentanecarboxylic acid. The isolation of this compound may throw light on the mechanism of the oxidation of *cyclo*hexanol (cf. Waters, *Ann. Reports*, 1945, **42**, 147; Milas, Harris, and Pangiotakos, *J. Amer. Chem. Soc.*, 1939, **61**, 2430).


(XI.)

5-Bromopentanecarboxylic acid was converted into the appropriate esters as follows:



The 7-fluoroheptanecarboxylates were synthesised from hexamethylene dibromide according to the following scheme. All the *compounds* beyond hexamethylene dibromide are new.

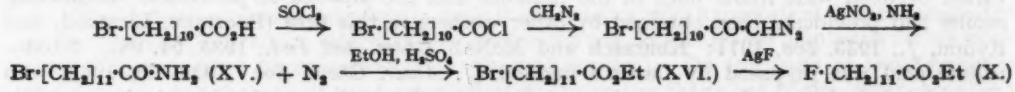


The splitting of (XII) took place very smoothly with constant-boiling hydriodic acid to give the pure iodo-acid. The fluorination of (XIII) was more facile than that of (XIV). In fact with the latter acid, hydrogen iodide was eliminated to some extent with the production of ethyl hept-6-enecarboxylate, which was effectively removed only by conversion into the dibromide with bromine, followed by distillation.

Ethyl and *2-fluoroethyl 9-bromononanecarboxylate* and ethyl and 2-fluoroethyl 9-fluorononanecarboxylate were all prepared from 9-bromononanecarboxylic acid, made by the action of hydrogen bromide and sulphuric acid on 9-acetoxynonanecarboxylic acid, which in turn was obtained by a four-stage synthesis from sebacic acid.

Ethyl 10-fluorodecanecarboxylate was readily prepared by the fluorination of the corresponding bromo-ester, prepared by esterifying the acid with ethyl alcohol and sulphuric acid.

Ethyl 11-fluoroundecanecarboxylate was synthesised from 10-bromodecanecarboxylic acid as follows:



The alcoholysis of (XV) to (XVI) was effected by boiling with absolute alcohol and sulphuric acid for 10 hours. The standard technique was adopted for the fluorination.

Experimental.

Ethyl δ-Bromovalerate.—δ-Bromovaleric acid (29 g., 0·16 mol.) was heated under reflux at 110° on an oil-bath for 8 hours with absolute alcohol (60 g., 1·2 mols.) and concentrated sulphuric acid (3 c.c.); the mixture was allowed to cool, diluted with water, extracted with ether, the ethereal solution dried (Na_2SO_4), and the product distilled under reduced pressure after removal of the ether; b. p. 104—106°/13 mm.; yield 27 g. (79%).

Reaction between Silver Fluoride and Ethyl δ-Bromovalerate.—Silver fluoride (17 g., 1·34 mols., dried over P_4O_{10} at 100°) was ground to a fine powder, and added slowly to ethyl δ-bromovalerate (14 g., 0·68 mol.) in a flask cooled in ice. A white cloudiness soon appeared, and the mixture was heated in a water-bath at 50° for 10 minutes, and kept at room temperature for 3 hours, with occasional shaking, by which time an orange coloration had developed. The mixture was diluted with ether, filtered, and distilled under reduced pressure. Impure ethyl δ-fluorovalerate (about 1 g.) was obtained as a colourless liquid, b. p. 70—88°/12 mm. (Found: F, 8·7. Calc. for $C_7H_{13}O_2F$: F, 12·87%).

Ethyl δ-Iodovalerate.—δ-Iodovaleric acid (60 g., 0·26 mol.) was heated on a sand-bath under reflux for 4 hours with an 8% solution of dry hydrogen chloride in absolute ethanol (300 c.c.). A deep red colour soon developed. The reaction mixture was cooled, diluted with an equal volume of water, extracted with ether, washed with sodium thiosulphate solution to remove iodine, dried (Na_2SO_4), the ether removed, and the product fractionated; yield 29·5 g.; b. p. 93—100°/13 mm. This was redistilled at 90—91°/12 mm. (Found: C, 50·8; H, 8·0. Calc. for $C_7H_{13}O_2I$: C, 32·8; H, 5·1%. Calc. for $C_7H_{13}O_2Cl$: C, 51·1; H, 7·9%).

Analysis for halogen by Stepanow's method assuming it to be chlorine gave Cl, 22·06 (Calc. for $C_7H_{13}O_2Cl$: Cl, 21·53%). It was therefore apparent that the Fischer–Speier method of esterification very readily substituted chlorine for iodine in the molecule as well as esterifying the acid; sulphuric acid was therefore used in place of hydrochloric acid in subsequent esterifications.

δ-Iodovaleric acid (36 g., 0·156 mol.) was heated under reflux on an oil-bath at 110° for 6 hours with absolute alcohol (84 c.c., 1·08 mols.) and concentrated sulphuric acid (3·6 c.c.). A deep red colour developed. The mixture was cooled, diluted with water, extracted with ether, shaken with sodium carbonate to remove excess of unchanged acid, and worked up as above. The ester (27 g., 69%) distilled at 108—118°/20 mm. (Found: I, 49·0. Calc. for $C_7H_{13}O_2I$: I, 49·2%).

Ethyl δ-Fluorovalerate.—Silver fluoride (34 g., 0·26 mol.) was slowly added (1 hour) with shaking to ethyl δ-iodovalerate (34 g., 0·13 mol.), the reaction flask being cooled in ice. After all the silver fluoride had been added, the flask was heated on a water-bath at 45—50° for 20 minutes, cooled, dry ether added, and the product filtered. The ethereal filtrate was washed with water to remove hydrogen fluoride, dried (Na_2SO_4), filtered, the ether removed, and the residue fractionated. Three fractions were collected: (1) b. p. 35—70°/10 mm., *ca.* 3 c.c.; (2) b. p. 70—90°/10 mm., *ca.* 2 c.c.; (3) b. p. 90—115°/10 mm. (mainly 110—115°), *ca.* 8 c.c. (recovered iodovalerate). Fraction (3) was refluorinated with silver fluoride (8 g.) by the same procedure as before. The fraction of b. p. 35—70°/10 mm. was collected (2 c.c.) and mixed with fraction (1) from the initial fluorination. The mixture was refractionated and a well-defined fraction of b. p. 56—60°/16 mm. (1·5 c.c.) was collected. The *ethyl δ-fluorovalerate* thus obtained was a colourless liquid with a pleasant fruit-like odour (Found: F, 12·53. $C_7H_{13}O_2F$ requires F, 12·87%).

5-Hydroxypentanecarboxylic Acid (cf. Robinson and Smith, *J.*, 1937, 373; Barger, Robinson, and Smith, *ibid.*, p. 718).*—To water (240 c.c.) and sulphuric acid (710 c.c.), cooled with stirring to below 15°, potassium persulphate (500 g.) was added, followed by alcohol (1000 c.c.), the temperature throughout being kept below 15°. The mixture was cooled to 10° and *cyclo*hexanone (100 c.c.) in alcohol (150 c.c.) was added during 2½ hours. After the addition, the stirring was continued at 10° for 15 minutes, and then for one hour without the cooling-bath. The mixture was then diluted to 7 l., filtered, saturated with ammonium sulphate, filtered again, and finally thoroughly extracted with ether. The extract was dried (Na_2SO_4), and the ether distilled off completely on a steam-bath. The residue was cooled and filtered if necessary from the solid (m. p. 130°) which may have separated out. The residue in the flask was 5-hydroxypentanecarboxylic acid (and traces of its lactone) and was sufficiently pure for the next stage.

5-Bromopentanecarboxylic Acid.—The above hydroxy-acid was added to a cold mixture of constant-boiling hydrobromic acid (550 c.c.) and sulphuric acid (125 c.c.) contained in a flask. The mixture was kept at room temperature for 2 hours with occasional shaking. The flask was then fitted with a reflux condenser and heated on a steam-bath for 6 hours. The product was then cooled, diluted with an equal volume of water, saturated with ammonium sulphate, and extracted with ether. After drying (Na_2SO_4), the ether was distilled off, and the residue consisted of the required acid. (If the acid were required pure it could be distilled at 160°/14 mm., but this was not necessary in preparing the ethyl and the 2-fluoroethyl ester.)

Ethyl 5-Bromopentanecarboxylate.—The foregoing crude bromo-acid was esterified with ethyl alcohol (108 c.c.) and sulphuric acid (5·3 c.c.) for 10 hours in an oil-bath at 110°. The product was cooled, diluted with an equal volume of water, and extracted with ether. The extract was dried (Na_2SO_4), the ether distilled off, and the residue fractionated, the fraction of b. p. 120—130°/15 mm. being collected; yield 61 g. (*i.e.*, 30% based on the *cyclo*hexanone used).

Ethyl 5-Fluoropentanecarboxylate.—The above ester (10 g., 0·045 mol.) was placed in a flask fitted with a reflux condenser and calcium chloride tube, and finely-powdered silver fluoride (11·4 g., 0·09 mol.) was added. A slight precipitate of silver bromide was produced almost immediately. The flask was heated to 50° for 30 minutes and then cooled. The contents were extracted with ether, and the extract filtered and dried (Na_2SO_4). The ether was distilled off, and the residue distilled under reduced pressure.

* Soon after the completion of our work (Report by Saunders to Ministry of Supply Meeting, London, June 1, 1944), Brown and Partridge (*J. Amer. Chem. Soc.*, 1944, **66**, 839) described similar modifications which confirmed our findings.

The fraction of b. p. 80—85°/14 mm. was redistilled, and the clear colourless *fluoro*-ester, b. p. 82—84°/14 mm., was collected; yield of twice distilled material, 2 g. (27%) (Found: C, 59·3; H, 9·5; F, 11·2. $C_8H_{15}O_2F$ requires C, 59·2; H, 9·25; F, 11·7%).

2-Fluoroethyl 5-Bromopentanecarboxylate.—5-Bromopentanecarboxylic acid was purified by distillation (b. p. 165—170°/20 mm.) and after recrystallisation from light petroleum had m. p. 35°. This acid (38 g.), fluoroethyl alcohol (30 g.), and sulphuric acid (2·5 c.c.) were thoroughly mixed and heated under reflux in an oil-bath at 120° for 5—6 hours. The mixture was cooled, diluted with water, and extracted with ether, and the extract dried (Na_2SO_4). After distillation of the ether, most of the residue came over at 144—146°/14 mm.; yield 25·1 g. (53%). Refractionation gave the *2-fluoroethyl* ester as a colourless liquid, b. p. 142°/13 mm., containing fluorine (Found: Br, 33·7. $C_9H_{14}O_2BrF$ requires Br, 33·2%).

This ester could also be prepared satisfactorily from unpurified bromo-acid. The crude bromo-acid (from *cyclo*hexanone 95 g., 1 mol.) was mixed with fluoroethyl alcohol (120 c.c.) and concentrated sulphuric acid (5 c.c.) and heated to 110—115° for about 10 hours. The product was cooled, diluted with an equal volume of water, and extracted with ether. The ether extract was dried (Na_2SO_4) and after distillation of the ether, the fraction of b. p. 138—143°/13 mm. was collected; yield 70 g. from 95 g. of *cyclo*hexanone (*i.e.*, 30%).

2-Fluoroethyl 5-Fluoropentanecarboxylate.—The foregoing bromo-ester (20 g., 0·083 mol.) and silver fluoride (21·6 g., 0·17 mol.) were kept at room temperature with occasional shaking for 15 minutes, then warmed to 40° for an hour with shaking. The product was cooled, extracted with ether, and the extract filtered, washed with an equal volume of water, and dried (Na_2SO_4). The subsequent fractionation was carried out in an atmosphere of nitrogen or dry carbon dioxide, by aid of a water-pump. The following fractions were obtained: (1) up to 106°/14 mm.; (2) 106—115/14 mm.; (3) 115—120°/14 mm. Fractions (2) and (3) were combined and refluorinated using silver fluoride (8 g.). The product was again extracted with ether, washed with water, dried, and distilled in an atmosphere of dry carbon dioxide. The following fractions were obtained: (1) 85—105°/12 mm.; (2) 107—108°/12 mm.; (3) 109—120°/12 mm. Fraction (2) was finally redistilled in an atmosphere of dry carbon dioxide, and the *2-fluoroethyl 5-fluoropentanecarboxylate*, b. p. 103—105°/14 mm., was collected as a colourless liquid (Found: F, 19·5. $C_8H_{14}O_2F_2$ requires F, 21·0%).

Investigation of the Solid of m. p. 130°.—The solid (see p. 1475) was recrystallised to constant m. p. from methanol. It was a colourless, odourless, crystalline material, insoluble in water, alkali, and hydrochloric acid. It contained no nitrogen, halogen, or sulphur. It reacted vigorously with concentrated sulphuric acid in the cold, with much charring, and it liberated iodine when boiled with acidified potassium iodide solution [Found: C, 63·4; H, 8·52; O, 28·08 (by diff.). $C_7H_{12}O$ requires C, 63·2; H, 8·77; O, 28·03%]. The molecular weight was determined in a semimicro-ebullioscopic apparatus (Found: *M*, 216, 223. $C_{14}H_{20}O_4$ requires *M*, 228). From the positive peroxide test by means of potassium iodide, it is very probable that the compound is *dicyclohexylidene peroxide* (XI).

6-Phenoxyhexyl Bromide.—Hexamethylene dibromide (244 g., 1 mol.), water (400 c.c.), and phenol (77 g., 0·82 mol.) were placed in a 2-l. 3-necked flask fitted with stirrer, dropping-funnel, and reflux condenser. The stirrer was started, the mixture heated to boiling, and 30% sodium hydroxide (106 c.c.) added through the dropping-funnel during 20 minutes. The mixture was heated under reflux for 8 hours with vigorous stirring, and allowed to cool. The upper layer was discarded, and the lower layer fractionated under reduced pressure, two fractions being obtained: (1) up to 174°/13 mm., mostly recovered hexamethylene dibromide; (2) 174—180°/13 mm. (112 g., 79% net), *6-phenoxyhexyl bromide* (Found: Br, 31·16. $C_{12}H_{17}OBr$ requires Br, 31·13%). The bromide was obtained as a colourless viscous liquid with a faint phenolic smell. The first fraction was shaken with 10% sodium hydroxide to remove phenol, and dried ($CaCl_2$). On distillation 100 g. of hexamethylene dibromide were recovered.

Ethyl 6-Phenoxyhexylmalonate.—Sodium (9·5 g., 0·42 atom) was dissolved in absolute alcohol (150 c.c., dried over magnesium ethoxide) in a 1-l. flask fitted with reflux condenser, stirrer, and dropping-funnel, and ethyl malonate (67 g., 0·42 mol.) was added with stirring. 6-Phenoxyhexyl bromide (108 g., 0·42 mol.) was slowly added with stirring, and after the addition was complete, the mixture was gently heated under reflux with stirring for 3 hours, during which a heavy white precipitate of sodium bromide was formed. The flask and contents were allowed to cool, water was added to dissolve the sodium bromide, and the non-aqueous layer was collected in ether and dried (Na_2SO_4). After removal of the ether, alcohol, and malonic ester with a water pump, the residual colourless viscous *ethyl 6-phenoxyhexylmalonate* was distilled by use of a mercury pump; yield 81 g. (51%); b. p. 155—158°/2 × 10^{-3} mm. (Found: C, 67·8; H, 8·46. $C_{19}H_{28}O_5$ requires C, 67·7; H, 8·34%).

6-Phenoxyhexylmalonic Acid.—The above ester (80 g., 0·24 mol.) was heated under reflux on an oil-bath with 20% sodium hydroxide solution (250 c.c.) until the mixture was homogeneous (4 hours); it was then cooled, and acidified strongly with dilute sulphuric acid. The thick white precipitate formed was filtered off, washed with a small quantity of cold water, recrystallised from water, separated, and dried in a vacuum over phosphoric oxide. The *acid* crystallised in short white needles (60 g., 88%), m. p. 162—163° (decomp.) (Found: C, 63·97; H, 7·06. $C_{15}H_{20}O_5$ requires C, 64·28; H, 7·14%).

7-Phenoxyheptanecarboxylic Acid.—6-Phenoxyhexylmalonic acid (60 g., 0·23 mol.) was heated in a round-bottom flask at 230°, with occasional stirring, until no more effervescence took place (2½ hours). The liquid so obtained solidified on cooling and was broken up, and recrystallised (animal charcoal) from light petroleum (b. p. 60—80°), forming colourless plates; yield of purified *acid*, 35 g. (58%); m. p. 69—70° (Found: C, 71·6; H, 8·6. $C_{14}H_{20}O_3$ requires C, 71·2; H, 8·47%).

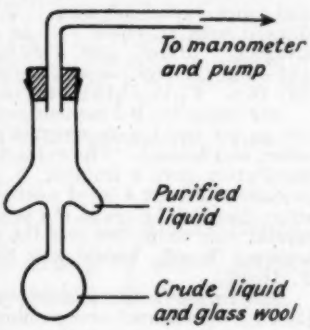
7-Iodoheptanecarboxylic Acid.—The phenoxy-acid (5 g., 0·021 mol.) was heated under reflux on an oil-bath at 160—170° with constant-boiling hydriodic acid (20 c.c., redistilled; *d* 1·7) for 6 hours. The product was cooled, extracted three times with ether, and the ethereal solution extracted twice with saturated sodium carbonate, this serving the double purpose of destroying the free iodine present and extracting the iodo-acid as its sodium salt. The aqueous solution so obtained was acidified with concentrated hydrochloric acid, and the white granular precipitate formed was filtered off, dried over phosphoric oxide, and recrystallised from light petroleum (b. p. 60—80°). The *iodo-acid* crystallised

in colourless rosettes (4 g., 70%), m. p. 43—44° (Found: I, 47·23. $C_8H_{13}O_2I$ requires I, 47·04%).

The experiment was repeated on a larger scale using 28 g. (0·12 mol.) of the phenoxy-acid, and 110 c.c. of constant-boiling hydriodic acid. The total yield of iodo-acid obtained in the two experiments was 27 g. (71%).

Ethyl 7-Iodoheptanecarboxylate.—The above acid (12 g., 0·44 mol.) was dissolved in absolute ethanol (80 g., 1·77 mols.), concentrated sulphuric acid (5·5 g., 0·054 mol.) was added, and the mixture was heated under reflux for 6 hours on an oil-bath at 110—120°. The product, which had become red, was cooled and poured into a large volume of water. The oil which separated was collected in ether, and the aqueous solution extracted twice with ether. The combined ethereal extracts were shaken twice with saturated sodium carbonate solution to remove iodine and hydriodic acid, then dried (Na_2SO_4) and distilled under reduced pressure. The *ethyl* ester was obtained as a colourless, mobile liquid (11·2 g., 83%) with a faint pleasant smell; b. p. 114°/1 mm. (Found: I, 42·6. $C_{10}H_{17}O_2I$ requires I, 43·0%).

Ethyl 7-Fluoroheptanecarboxylate.—Silver fluoride (9·7 g., 0·076 mol.) was added slowly to the iodo-ester (10·2 g., 0·034 mol.) in a 50-c.c. round-bottom flask. The mixture slowly became warm and a yellow precipitate began to form. The mixture was continuously stirred, and the silver halide ground to expose fresh silver fluoride to the iodo-ester. It was not necessary to cool the reaction vessel, and after 30 minutes the heavy yellow precipitate of silver iodide which had been formed turned brick-red, presumably owing to formation of silver iodofluoride. The reaction mixture was cooled in ice and extracted with dry ether, and the extract washed with water, dried (Na_2SO_4), and distilled under reduced pressure. A fraction was collected of b. p. 100—108°/12 mm. (2 g.), which contained fluorine and decolorised bromine-water in the cold, indicating the presence of an olefinic bond (Found: F, 5·7. Calc. for $C_{10}H_{17}O_2F$: F, 10·0%). It appeared that a considerable proportion of ethyl hept-6-ene-carboxylate had been formed during the fluorination. As this ester would be very hard to remove from the fluoro-ester by fractional distillation owing to similarity of b. p., it was decided to brominate the olefinic ester, for the dibromo-ester should have a much higher b. p. than the desired fluoro-ester. Bromine (dried with concentrated sulphuric acid) in carbon tetrachloride was therefore added dropwise to the ester mixture, until a faint brown colour just remained. The carbon tetrachloride and excess of bromine were then removed by distillation, and the residual colourless liquid (*ca.* 1 g.) was distilled. The volume of liquid was too small to allow the use of a normal distillation flask, so the method described below was adopted. The distillation flask used was as shown in the diagram, the liquid to be distilled being placed in the bulb (of about 3 c.c. capacity) with glass-wool. The bulb was placed in an oil-bath, and the apparatus connected to a vacuum pump. When the oil-bath was heated, the liquid distilled into the upper, pear-shaped bulb from which it could be removed. (The efficiency of the flask had first been determined by distillation of a 4 : 1 mixture of benzene and toluene.) The b. p. of each fraction was determined by the method of Emich (*Monatsh.*, 1917, **38**, 219).



Three fractions were obtained from the crude *ethyl 7-fluoroheptanecarboxylate*. The third fraction (0·2 g.) distilled at an oil-bath temperature of 145—150°/12 mm., and contained fluorine. The ester was thus obtained as a colourless mobile liquid with a pleasant fruity smell; b. p. 191° (Found: C, 63·1; H, 9·9. $C_{10}H_{17}O_2F$ requires C, 63·2; H, 10·0%).

2-Fluoroethyl 7-Iodoheptanecarboxylate.—7-Iodoheptanecarboxylic acid (13 g., 0·48 mol.) was dissolved in 2-fluoroethyl alcohol (110 g., 1·73 mol.), and concentrated sulphuric acid (5·5 g., 0·054 mol.) was added. The mixture was heated under reflux for 6 hours on an oil-bath at 120—130°. The product was allowed to cool, poured into a large volume of water, and the deep red, oily, lower layer which separated was run off. The aqueous solution was twice extracted with ether, and the ethereal extracts united with the crude ester. The extract was washed twice with 20% sodium carbonate solution, and dried (Na_2SO_4). After filtration, and removal of the ether, the residual oil was distilled. The *fluoroethyl* ester was obtained as a colourless mobile liquid (9·5 g., 62·5%) with a very faint pleasant odour; b. p. 122—124°/0·8 mm. (Found: I, 39·9. $C_{10}H_{16}O_2IF$ requires I, 40·2%).

2-Fluoroethyl 7-Fluoroheptanecarboxylate.—Silver fluoride (9 g., 0·07 mol.) was added slowly to the foregoing ester (8·5 g., 0·027 mol.) in a 50-c.c. round-bottom flask. The mixture slowly became warm, with simultaneous formation of a yellow precipitate; it was stirred with a glass rod, and the silver halide ground continuously to expose fresh silver fluoride. After about 30 minutes the precipitate became red, and dry ether was added. The mixture was filtered, and the heavy precipitate thoroughly extracted with dry ether. The ethereal extract was washed with water to remove hydrofluoric acid, dried (Na_2SO_4), the ether removed, and the residual oil distilled, the fraction of b. p. 128—130°/13 mm. being collected (1·1 g., 21%). *2-Fluoroethyl 7-fluoroheptanecarboxylate* was obtained as a colourless mobile liquid with a pleasant fruit-like smell, very similar to that of the ethyl ester (Found: F, 17·84. $C_{10}H_{16}O_2F_2$ requires F, 18·27%).

9-Bromononanecarboxylic Acid.—Sebacic acid (202 g., 1·0 mol.) and ethyl sebacate (150 g., 0·58 mol.) were converted into ethyl hydrogen sebacate by the method of Swann, Oehler, and Buswell (*Org. Synth.*, Coll. Vol. II, 276); yield 147 g. (65%). The latter ester (115 g., 0·5 mol.) was converted into crystalline potassium ethyl sebacate in theoretical yield (134 g.). This salt (134 g.) was reduced to crude 9-hydroxynonanecarboxylic acid by the method of Grun and Wirth (*Ber.*, 1922, **55**, 2208). The crude product, which contained sebacic acid, was heated in a flask to 100°, and acetic anhydride (250 c.c.) was added a few c.c. at a time as a vigorous reaction took place after each addition. The contents of the flask were cooled and diluted with water (2 vols.), and the solution extracted three times with light petroleum (b. p. 60—80°). After drying (Na_2SO_4), and removal of solvent, the residue consisted of crude 9-acetoxynonanecarboxylic acid. This was shaken with a cooled mixture of constant-boiling hydrobromic acid (400 c.c.) and concentrated sulphuric acid (96 c.c.), and set aside overnight. The mixture

was then heated on a water-bath for 4 hours, cooled, diluted with an equal volume of water, and extracted with ether. The extract was dried (Na_2SO_4) and after removal of ether, the residue was distilled and 9-bromononanecarboxylic acid was obtained as a colourless liquid, b. p. 145—155°/0·4 mm. It solidified to a colourless solid, m. p. 42° (yield 15 g.; 12% based on the ethyl hydrogen sebacate used). This acid had been prepared by a different method by Chuit and Hausser (*Helv. Chim. Acta*, 1929, **12**, 474), who gave m. p. 42°, b. p. 163—165°/2 mm.

Ethyl 9-*Bromononanecarboxylate*.—The bromo-acid (12·5 g., 0·05 mol.), ethyl alcohol (15 c.c.), and concentrated sulphuric acid (2·0 c.c.) were heated under reflux for 2 hours, cooled, diluted with an equal volume of water, and extracted with ether. The extract was dried (Na_2SO_4), the ether removed, and the residue distilled under reduced pressure. The *ethyl* ester distilled at 162—164°/10 mm. as a colourless liquid (10 g., 72%) (Found: Br, 28·7. $C_{13}H_{25}O_2Br$ requires Br, 28·7%).

Ethyl 9-*Fluorononanecarboxylate*.—The bromo-ester (10 g., 0·036 mol.) and pure, dry, finely-powdered silver fluoride (10 g., 0·08 mol.) were warmed to 80° for 20 minutes, during which a yellow precipitate of silver bromide separated. The contents of the flask were cooled and extracted with ether. The extract was washed and dried (Na_2SO_4). The ether was removed, and the residue on distillation yielded the following fractions: (1) 130—145°/10 mm.; (2) 145—150°/10 mm.; (3) 150—165°/10 mm. All three fractions contained fluorine. The first fraction contained no bromine, but the second and third contained traces. The two higher fractions were accordingly refluorinated with silver fluoride (4 g.), and, after isolation as above, were combined with the first fraction and the whole redistilled. The *ethyl* 9-*fluorononanecarboxylate*, b. p. 135—138°/10 mm., was collected as a colourless, pleasant-smelling liquid (1·5 g., 20%) (Found: F, 8·4. $C_{13}H_{25}O_2F$ requires F, 8·7%).

2-*Fluoroethyl* 9-*Bromononanecarboxylate*.—The acid (10 g., 0·04 mol.), fluoroethyl alcohol (20 c.c.), and concentrated sulphuric acid (2·0 c.c.) were heated under reflux for 1 hour. The mixture was cooled, diluted with an equal volume of water, and extracted 3 times with ether. The extract was dried (Na_2SO_4), filtered, and distilled. After removal of the ether, nearly all the *ester* came over at 184—188°/11 mm., as a colourless liquid (10·4 g., 87%) melting just below room temperature (Found: Br, 26·6. $C_{13}H_{25}O_2BrF$ requires Br, 26·9%).

2-*Fluoroethyl* 9-*Fluorononanecarboxylate*.—The bromo-ester (9·4 g., 0·03 mol.) and silver fluoride (13 g., 0·1 mol.) were warmed at 75° for 15 minutes. The mixture was then cooled, extracted with ether, and filtered. The extract was washed and dried (Na_2SO_4), the ether removed, and the residue on distillation gave a fraction, b. p. 142—150°/12 mm., which contained fluorine and a slight trace of bromine. About 4 g. of unchanged bromo-ester were recovered. This was fluorinated by means of silver fluoride (5 g.), and the product isolated as before and added to the above fraction. The combined liquids were redistilled and the *ester* of b. p. 145—149°/12 mm. was collected as a colourless, pleasant-smelling liquid, which was bromine-free (1·3 g., 17%) (Found: F, 14·6. $C_{13}H_{25}O_2F_3$ requires F, 16·0%).

Ethyl 10-*Bromodecanecarboxylate*.—The corresponding acid (10 g., 0·038 mol.) and ethyl alcohol (10 c.c.) were heated under reflux with concentrated sulphuric acid (1·0 c.c.) for 1½ hours. The mixture was cooled, an equal volume of water added, and the whole extracted three times with ether. The extract was dried (Na_2SO_4). After removal of the ether, the *ester* distilled at 137°/0·15 mm. (9·0 g., 81%) (Found: Br, 27·0. $C_{13}H_{25}O_2Br$ requires Br, 27·2%).

Ethyl 10-*Fluorodecanecarboxylate*.—The bromo-ester (8 g., 0·027 mol.) and silver fluoride (8 g., 0·06 mol.) were heated at 75° with constant shaking. Separation of silver bromide occurred almost immediately and after 30 minutes the reaction appeared to be complete. The product was cooled, extracted with ether, filtered, and the ethereal extract washed with water. After drying (Na_2SO_4) and removal of the ether, the *fluoro*-ester, b. p. 82—84°/0·02 mm., 140—141°/11 mm., was obtained (1·2 g., 19%) (Found: F, 7·8. $C_{13}H_{25}O_2F$ requires F, 8·2%).

10-*Bromodecanecarboxyl Chloride*.—The corresponding acid (26·5 g., 0·1 mol.) and pure thionyl chloride (18 g., 0·15 mol.) were heated on a boiling water-bath for 1½ hours. The mixture was then directly distilled under reduced pressure. The *acid chloride* was obtained as a colourless liquid, b. p. 174—175°/10 mm. (23·5 g., 83%) (Found: total halogen, 40·7. $C_{11}H_{20}OClBr$ requires total halogen, 40·6%).

10-*Bromo*-1-*diazoacetyldecane*.—The foregoing chloride (9·1 g., 0·033 mol.) in dry ether was added to diazomethane (8·4 g., 0·2 mol.) in dry ether (350 c.c.) with mechanical stirring and efficient cooling in a tared flask at 0—5°. The solution was kept overnight in a refrigerator, and the *diazo-ketone* was isolated by evaporation under reduced pressure, a water-bath at a temperature of less than 30° being used. It was obtained as a yellow solid, m. p. *ca.* 30° (12·5 g., 98%).

11-*Bromoundecanecarboxyamide*.—The diazo-ketone (12·5 g., 0·043 mol.) was dissolved in warm, freshly-distilled dioxan (100 c.c.) and then treated with 20% aqueous ammonia (30 g., 0·35 mol.) and 10% aqueous silver nitrate (6 c.c.) under reflux in a large flask on a boiling water-bath. A vigorous effervescence occurred at once, and the clear yellow solution turned brown and opaque. The solution was heated for 25 minutes and then filtered hot. On cooling, a colourless, micro-crystalline solid was deposited. This was recrystallised from aqueous alcohol (charcoal). The pure *amide* (5·7 g., 49%) had m. p. 105°, and evolved ammonia on being heated with soda–lime (Found: N, 5·24. $C_{12}H_{24}ONBr$ requires N, 5·04%).

Ethyl 11-*Bromoundecanecarboxylate*.—The above amide (3·5 g., 0·013 mol.) was heated under reflux with absolute ethyl alcohol (20 c.c.) and concentrated sulphuric acid (6 c.c.) for 10 hours in an oil-bath at 130—140°. The product was cooled and extracted three times with ether. The extracts were thoroughly washed with water and dried (Na_2SO_4). After removal of the ether, the residue was fractionally distilled and the *ester* was obtained as a clear colourless liquid (1·8 g., 47%), b. p. 130—132°/$2·5 \times 10^{-2}$ mm. Redistillation gave a b. p. of 127°/9×10^{-3} mm. (Found: Br, 25·6. $C_{14}H_{27}O_2Br$ requires Br, 26·0%).

Ethyl 11-*Fluoroundecanecarboxylate*.—The bromo-ester (5 g., 0·017 mol.) and silver fluoride (5 g., 0·039 mol.) were gently heated in a water-bath at 80° for 15 minutes. Silver bromide was formed. The mixture was cooled and extracted with ether, and the ethereal extracts were washed twice with

water and dried (Na_2SO_4). After removal of the ether, the residue was fractionated, yielding a colourless liquid, b. p. 151°/11 mm., which contained F and no Br. The higher fraction, b. p. 155—180°/11 mm., was refluorinated by means of silver fluoride (3 g., 0·023 mol.) and 25 minutes' heating at 90°. The product was isolated as before and dried (Na_2SO_4). To the residue, after removal of the ether, was added the fraction of b. p. 151°/11 mm., and the combined liquids were fractionated. The *fluoro*-ester was obtained as a clear, colourless liquid (0·5 g., 12%), b. p. 152—153°/11 mm. (Found: F, 7·3. $C_{14}H_{27}O_2F$ requires F, 7·7%).

Preparation of Silver Fluoride.—Commercial silver fluoride was usually found to be unsuitable for the fluorinations mentioned in this paper. It was therefore prepared according to Moissan's method (*Bull. Soc. chim.*, 1891, **5**, 456, 880) from freshly prepared silver carbonate (from 106 g. of anhydrous sodium carbonate and 340 g. of silver nitrate) and 40% hydrofluoric acid (100 g.). The reaction was carried out in a large platinum dish. Recrystallisation from water was carried out in the dark and gave silver fluoride which contained water. In order to remove the last traces of water two methods were found to be successful: (*a*) The crystals were added to three times their volume of pure, dry benzene, which was then slowly distilled: the water–benzene azeotrope distilled first, and then pure benzene; the last traces of liquid were finally removed by connecting the distilling-flask to a water-pump and heating it to 140°. (*b*) The crystals were dried for several weeks in a vacuum over fresh phosphoric oxide in the dark. The former method had the advantage of speed. The pure silver fluoride was obtained as a brown powder, which was finely ground in a mortar before use. It was stored in a bottle covered with black paper. The yield varied between 125 g. and 150 g. (50—60% based on silver nitrate used).

We wish to thank the Chief Scientist of the Ministry of Supply for permission to publish details of the work concerning 5-fluoropentanecarboxylic acid and its derivatives. The remainder of the work was subsidised by maintenance grants to two of us (F. J. B. and F. L. M. P.) made by I.C.I. General Chemicals (Widnes), to whom we express our gratitude. Our thanks are also due to Prof. Chibnall for a sample of 10-bromodecanecarboxylic acid. We are grateful to Mr. K. Carpenter and Dr. W. J. O'Connor for the majority of the toxicity tests on small animals.

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315. *The Reaction of Cyanogen and Related Nitriles with* 1 : 3-*Dienes. Part I. Thermodynamic Considerations, and a Study of the Uncatalysed Reaction of Cyanogen and Related Nitriles with* 1 : 3-*Butadiene.*

By P. J. HAWKINS and G. J. JANZ.

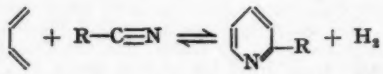
Some thermodynamic calculations and an experimental study of the reaction of butadiene with cyanogen, hydrogen cyanide, acetonitrile, and benzonitrile are reported. Although these reactions are in each case quite favoured energetically, the conversions were quite small, except in the case of cyanogen. These reactions may be considered as further examples of the Diels–Alder reaction, in which the C≡N group is the dienophilic component, and in which the intermediate 2 : 5-dihydropyridine derivatives lose hydrogen under the conditions of the reaction to form the corresponding pyridine derivatives.

REACTION of cyanogen with butadiene in the gaseous phase at moderately high temperatures leads to formation of 2-cyanopyridine (Janz, Ascah, and Keenan, *Canadian J. Res.*, 1947, **25**, B, 272). Further study established that this behaviour may be generalised for the simple 1 : 3-dienes such as isoprene, chloroprene, and piperylene, the product in each case being a 2-cyanopyridine derivative (Janz and Keenan, *ibid.*, p. 283). It was suggested that the reaction may be explained as proceeding by a Diels–Alder mechanism, in which the C≡N has the rôle of the dienophilic component of the dienophile, and that because the conditions are favourable for aromatisation, the reaction is accompanied by a simultaneous dehydrogenation, giving the 2-cyanopyridine. This is in accord with some observations by Alder ("Preparative Organic Chemistry," Interscience Publishers Inc., N.Y., 1948) that, when derivatives of 2 : 5-dihydropyridines are formed in the Diels–Alder reaction, the substances lose hydrogen under the normal conditions of the reactions to form the corresponding pyridine derivatives.

Further studies are now reported of the application of the diene synthesis to the formation of pyridinic nuclei, with particular reference to the use of nitriles other than cyanogen. The nitriles chosen, hydrogen cyanide, benzonitrile, and acetonitrile may be considered as members of the general class of dienophiles, R·C⋮N. Apart from the papers already mentioned, very little has been published on this class of dienophile. Dilthey *et al.* (*Ber.*, 1935, **68**, 1162) reported the synthesis of pentaphenylpyridine from benzonitrile and tetraphenyl*cyclo*pentadienone. This may be considered as a Diels–Alder reaction, accompanied by a simultaneous cleavage of carbon monoxide. The only other direct application is reported by Alder (*loc. cit.*), who used the esters of cyanoformic acid in the reaction with butadiene to form a corresponding pyridine derivative.

The thermodynamic approach was first considered in order to obtain information on the feasibility of these reactions over the temperature range to be investigated. Calculations concerning the hydrogenation of pyridine to piperidine were also undertaken to find the stability of the dihydro-intermediates with reference to the end products.

The method of calculating $\Delta G°$ and $\log K_p$ from the thermodynamic functions is well established, and $\Delta G°$ and $\log K_p$ were calculated by this method for the reactions considered except for the 2-phenylpyridine synthesis, for which, owing to lack of sufficient data to calculate the thermodynamic functions, the results were obtained by using the principles of the method of group contributions to estimate the necessary data (Andersen, Beyer, and Watson, *Nat. Pet. News*, 1944, **36**, R476). The reaction considered in each case was simply



Butadiene and Hydrogen Cyanide.—The thermodynamic functions for butadiene (Brickwedde, Moskow, and Aston, *J. Res. Nat. But. Stand.*, 1946, **37**, 263), hydrogen cyanide (Gordon, *J. Chem. Physics*, 1937, **5**, 30), pyridine (Kline and Turkevich, *ibid.*, 1944, **12**, 300), and hydrogen (Amer. Pet. Institute Project 44, Tables and Selected Values, Nat. Bur. Stand., Washington, D.C., 1946) have all been previously calculated from molecular and spectroscopic data, and were used to calculate $\Delta G°$ and $\log K_p$ for this reaction. The results are listed in Table II.

Butadiene and Acetonitrile.—The thermodynamic functions for 2-methylpyridine are listed in Table I, and are probably of sufficient accuracy for most thermodynamic calculations of the type considered. The spectroscopic frequency assignment for the calculation of the vibrational contribution to the thermodynamic function for 2-methylpyridine was made on a semi-empirical basis using the frequency assignment for pyridine (Kline and Turkevich, *loc. cit.*) as guide, and the frequencies associated with the vibrations of the methyl group. Strictly, it is incorrect to speak of the vibrations of an individual link or group, but in such a group as methyl, where a light atom moves against a much heavier residue, the vibrational frequencies associated with such vibrations are little changed from one molecule to another. By using the pyridine assignment, minus the frequencies 3054, 1061, and 747 (associated with the C–H in the 2-position of the pyridine molecule), and plus the frequencies 3000 (3), 1400 (3), 1000 (3), 1120 (2), 920 (for the methyl group in the 2-position), the vibrational contributions to the thermodynamic functions were calculated. For the rotational contribution, the product of the moments of inertia, ABC, for the molecule was calculated by using Kline and Turkevich's parameters for the pyridine nucleus and 1·40 and 1·087 A. for the C–C and C–H bond lengths, respectively, of the methyl group in the 2-position. ABC was thus found to be $1·8722 \times 10^{-113}$. The heat of formation of 2-methylpyridine, $\Delta H°_{298·1(g)} = 21·5$ kcals./mol., was calculated from the heat of combustion (Int. Critical Tables, Vol. 5) and the latest thermal data for carbon dioxide and water (Amer. Pet. Inst. Project 44, *loc. cit.*). By using the data for acetonitrile (Thompson, *Trans. Faraday Soc.*, 1941, **37**, 344), butadiene, and hydrogen, together with the above data, the $\Delta G°$ and $\log K_p$ values (Table II) were calculated.

Butadiene and Cyanogen.—The thermodynamic functions for 2-cyanopyridine have been calculated from molecular and spectroscopic data, as for 2-methylpyridine, and are listed in Table I. For the calculation of the vibrational contribution, the frequencies associated with the vibrations of the cyano-group in the 2-position, 300 (2), 380 (2), 900, 2245, were used in conjunction with the assignment for pyridine (Kline and Turkevich, *loc. cit.*), minus the frequencies associated with the C–H vibrations in the 2-position. The cyano-group has vibrational frequencies which generally remain fairly constant from one molecule to another, being sometimes slightly affected by neighbouring groups or electronic influences such as conjugation, and the values above were chosen from a consideration of the assignments made for other nitriles (Herzberg, "Infra-Red and Raman Spectra," D. Van Nostrand Co. Inc., N.Y., 1945; Kohlrausch, "Der Smekal–Raman Effekt," Julius Springer, Berlin, 1938). By using 1·15 A. for the C≡N bond length and the same parameters as for 2-methylpyridine, the product of the moments of inertia, ABC, was found to be $4·402 \times 10^{-113}$. The heat of formation of 2-cyanopyridine was estimated by using 2-methylpyridine as the parent, and the increment for the change of cyano- for methyl in the molecule. This gave $\Delta H°_{298·1(g)} = 60·59$ kcals./mol., and this value is recommended in preference to that reported in the earlier calculations for this compound (Janz, *Canadian J. Res.*, 1947, **25, B,** 331). The values of $\Delta G°$ and $\log K_p$ were calculated from these data, together with those for butadiene, hydrogen, and cyanogen (Thompson, *loc. cit.*) and are listed in Table II.

TABLE I.

Thermodynamic functions for 2-methylpyridine and 2-cyanopyridine.

Function.	$T°$, K.	298·1.	300.	400.	500.	600.	700.	800.	900.	1000.	1200.
$-(G° - E_0°/T)$											
2-Methylpyridine	.....	59·65	59·87	63·61	67·93	71·93	75·84	80·55	83·56	85·63	90·95
2-Cyanopyridine	.....	62·52	62·75	68·05	72·30	76·76	80·98	86·19	88·84	91·08	96·30
$(H° - E_0°)$											
2-Methylpyridine	.....	3·52	3·70	6·13	9·99	14·23	19·03	23·16	29·84	34·29	48·21
2-Cyanopyridine	.....	4·36	4·54	7·32	11·35	15·59	20·21	24·07	30·32	34·27	46·97
$C_p°$.											
2-Methylpyridine	.....	21·11	22·70	29·81	39·24	45·36	50·27	52·36	57·28	58·62	64·57
2-Cyanopyridine	.....	26·50	27·10	32·46	39·96	44·41	47·88	48·81	52·73	53·24	57·74

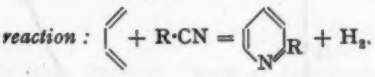
TABLE II.

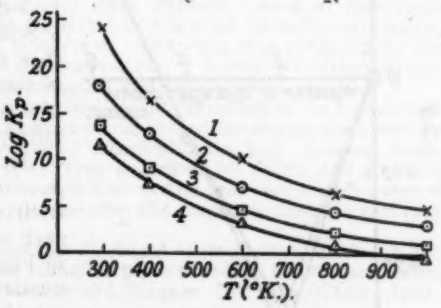
Free-energy change and log K_p for some reactions of butadiene and organic nitriles.

Reaction.	$T°$, K.	298·1.	400.	600.	800.	1000.
(i) $C_4H_6 + HCN = C_5H_5N + H_2$	$-(\Delta G°)$	−24·56	−22·97	−19·80	−16·73	−13·71
	log K_p	18·0	12·6	7·2	4·6	3·0
(ii) $C_4H_6 + CH_2{\cdot}CN = C_5H_4N{\cdot}CH_3 + H_2$	$-(\Delta G°)$	−18·77	−16·48	−12·26	− 8·49	− 2·47
	log K_p	13·8	9·0	4·5	2·3	0·5
(iii) $C_4H_6 + (CN)_2 = C_5H_4N{\cdot}CN + H_2$	$-(\Delta G°)$	−33·15	−31·76	−28·70	−27·02	−22·12
	log K_p	24·3	17·4	10·5	7·4	4·8
(iv) $C_4H_6 + C_6H_5{\cdot}CN = C_5H_4N{\cdot}C_6H_5 + H_2$ ...	$-(\Delta G°)$	−13·55	−13·91	− 9·37	− 3·83	0·99
	log K_p	11·9	7·6	3·4	1·05	− 0·2

Butadiene and Benzonitrile.—The thermal data for benzonitrile and 2-phenylpyridine have been estimated by the method of group contributions (Andersen, Beyer, and Watson, *loc. cit.*). For benzonitrile the parent hydrocarbon chosen was toluene, for which accurate thermal data are available (Amer. Pet. Inst. Project 44, *loc. cit.*). The increment due to cyano- for methyl was estimated by comparing the data for 2-methylpyridine and 2-cyanopyridine. Thus for benzonitrile, $\Delta H°_{298·1(g)} = 50·85$, $S°_{298·1} = 82·12$, and $C_p° = 3·12 + 84·59 \times 10^{-3}\ T - 30·75 \times 10^{-6}\ T^2$. In a similar manner the thermal data for 2-phenylpyridine were estimated by using the increment due to substitution of phenyl for methyl as given by Andersen *et al.*, together with 2-methylpyridine as the reference compound. This gives for 2-phenylpyridine, $\Delta H°_{298·1(g)} = 53·89$ kcals., $S°_{298·1} = 93·12$ cals. deg.$^{-1}$ mol.$^{-1}$, and $C_p° = -12·55 + 178·33 \times 10^{-3}\ T - 71·15 \times 10^{-6}\ T^2$. The log K_p and $\Delta G°$ calculated from these data are listed in Table II. These values are of necessity a rougher approximation than those calculated for the other examples, but they should serve for indicating the general trend in this series.

FIG. 1.

For the reaction :  + R·CN = + H_2.

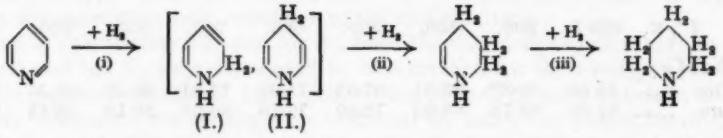


(1) R = CN; (2) R = H; (3) R = CH_3; (4) R = C_6H_5.

It is seen in Fig. 1 that log K_p decreases as the temperature increases in each example considered. It would be preferable to study the reactions at the lower temperatures where the thermodynamic conditions are more favourable. Over the whole temperature range considered, the free-energy change for the reaction (*i.e.*, thermodynamic stability with reference to the reactants) is greatest for the case of cyanogen, and decreases for hydrogen cyanide, acetonitrile, and benzonitrile, in that order. Temperatures higher than 800° K. are predicted to be not very favourable for the reaction involving benzonitrile (*i.e.*, log. $K_p \leq 0$).

Hydrogenation of Pyridine.—A consideration of the thermodynamics of the hydrogenation of benzene (unpublished) established that the dihydro- and tetrahydro-intermediates were thermodynamically unstable with reference to benzene and *cyclo*hexane. A similar result may

be expected in the case of pyridine. The following steps have been considered in the hydrogenation of pyridine:



(I.) (II.)

As well as the 1 : 2-dihydropyridine (I), the 1 : 4-dihydro-intermediate was considered, since there is evidence that under suitable conditions the hydrogenation will proceed by this path (Shaw, *J.*, 1924, **125**, 1930; 1925, **127**, 215; 1937, 300). The free-energy change was calculated from the relationship $\Delta G = \Delta H - T\Delta S$, where ΔH is the heat of hydrogenation, and ΔS the corresponding entropy change. The heats of hydrogenation and entropy changes for the separate steps are listed in Table III. The heat of hydrogenation of pyridine to piperidine

TABLE III.

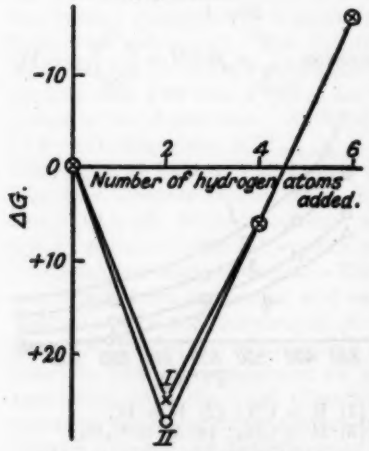
The hydrogenation of pyridine to piperidine.

Reaction.	$\Delta H_{298\cdot1}$, kcals.	$\Delta G_{298\cdot1}$, kcals.
Pyridine to 1 : 2-dihydropyridine	16·7	25·3
Pyridine to 1 : 4-dihydropyridine	18·5	27·1
1 : 2-Dihydro- to tetrahydro-pyridine	−28·2	−19·6
1 : 4-Dihydro- to tetrahydro-pyridine	−30·0	−21·4
Tetrahydropyridine to piperidine	−30·0	−20·0
Pyridine to piperidine	−40·1	−14·3

was calculated from the heats of formation based on the thermal data for these compounds (Int. Critical Tables) and for carbon dioxide and water (Amer. Pet. Inst. Project 44, *loc. cit.*).

FIG. 2.

Hydrogenation of pyridine to piperidine.



(I) 1 : 2-*Dihydropyridine*;
(II) 1 : 4-*dihydropyridine*.

The heat of hydrogenation for the double bond in tetrahydro- and 1 : 4-dihydro-pyridine was assumed to be the same as that for a true olefinic linkage (*idem, ibid.*) since no resonance energy change is involved. This value was corrected for the change in resonance energy, 1·8 kcals., in the hydrogenation of 1 : 2-dihydropyridine, where it was assumed that the resonance is the same as in *cyclo*hexa-1 : 3-diene (Wheland, "The Theory of Resonance," J. Wiley & Sons Inc., N.Y., 1944). The value for the first step was then calculated by difference. A reasonable approximation of the entropy of hydrogenation of pyridine to piperidine is obtainable from a consideration of the difference in entropy between benzene and *cyclo*hexane, and the entropy of formation of hydrogen (Amer. Pet. Inst. Project 44, *loc. cit.*). This gave $\Delta S = 86{\cdot}7$ cals. deg.$^{-1}$ mol.$^{-1}$ for this hydrogenation. The values for the free-energy change for each step calculated from these data (Table III) are plotted in Fig. 2 to show the thermodynamic stability of the intermediates with reference to the end products. In this calculation, because of the absence of values for the intermediate stages, it was assumed that the total entropy change is evenly divided over the three stages. It is seen that both the dihydro- and the tetrahydro-pyridine are thermodynamically unstable with reference to pyridine and piperidine, and even in the limits where it would be assumed that ΔS is zero or equal to $-86{\cdot}7$ cals. deg.$^{-1}$ mol.$^{-1}$ in the first step of the hydrogenation, the calculation predicts the dihydropyridine to be thermodynamically unstable relative to the other substances. Although only approximate, the results are of interest in view of the experimental observations concerning the present research, and the generalisation made by Alder (*loc. cit.*). If either of these intermediates was formed under conditions favouring aromatisation, one would expect it to pass over into pyridine.

These reactions were studied over the temperature range 400—600° and for various reaction contact times in an unpacked vessel. The catalysis of these reactions is described in Part II

(following paper). The data and results for the uncatalysed reactions are summarised in Table IV. It was found that when the reaction of butadiene with hydrogen cyanide, acetonitrile, benzonitrile, or cyanogen is carried out in this temperature range and at atmospheric pressure, the pyridinic product, *i.e.*, pyridine, or 2-methyl-, 2-phenyl-, or 2-cyano-pyridine, respectively, is formed in each case.

The space-time yields (mols. $\times 10^{-4}$ of pyridinic product/hr./100 c.c. reaction vessel volume) for these reactions are listed below. These results are for runs at 400° using comparable space velocities (0·3—0·5 l./hr./c.c. at 0° and 760 mm.) and molar ratios of nitrile to diene (approx. 3 : 1).

	Reaction of butadiene with :			
	Benzonitrile.	Acetonitrile.	Hydrogen cyanide.	Cyanogen.
Product	2-Phenylpyridine	2-Methylpyridine	Pyridine	2-Cyanopyridine
Space-time yield (mols. $\times 10^{-4}$/hr./100 c.c.)	1·45	1·88	3·0	16·7

The space-time yields are quite small, indicating that the reaction velocities are low. The relatively larger yield for the cyanogen–butadiene reaction is probably partly accounted for by the fact that the effective concentration of the cyano-group for this nitrile is double that for the same concentration of the other nitriles. The above results indicate that the reactions do not have very convenient velocities, and in view of the thermodynamic calculations predicting a very favourable free-energy change in this temperature range, a search for a catalyst to promote the reaction was undertaken (see Part II).

The space-time yield in the benzonitrile–butadiene reaction when the vessel was packed with Pyrex-glass chips was found to be about the same as when the unpacked reactor was used (cf. runs 24 and 6, Table IV, *d*). The reaction seems to be essentially homogeneous.

In the temperature range investigated, 400—600°, the conversions of butadiene into pyridinic product were all low (1% or less) when hydrogen cyanide, acetonitrile, and benzonitrile were used in the reaction. As a novel route to the synthesis of pyridine and 2-substituted pyridines other than 2-cyanopyridine (Janz, Ascah, and Keenan, *loc. cit.*) the uncatalysed reaction of butadiene with organic nitriles would not seem to be of much interest.

Experimental.

Materials.—Butadiene and hydrogen cyanide were samples from Imperial Chemical Industries Limited. The hydrogen cyanide was normally stored in glacial acetic acid solution (Krieble and Snellie, *J. Amer. Chem. Soc.*, 1945, **67**, 690), and freshly distilled before use. Cyanogen was prepared by the copper sulphate–sodium cyanide method (Janz, Ascah, and Keenan, *loc. cit.*). Acetonitrile (Hopkin and Williams) and benzonitrile (B.D.H.) were dried over phosphoric oxide and redistilled before use.

Flow Apparatus.—The design of the flow apparatus was essentially that described in the butadiene–cyanogen work (*idem, ibid.*). The reactors were of Pyrex glass or silica, and of the design described by Farkas and Melville (" Experimental Methods in Gas Reactions," MacMillan Co. Ltd., London, 1939, p. 302), and the liquid flow-meters were a refinement of spiral type described by Fabris and Peacock (*Canadian Chem. & Process Ind.*, Feb., 1947), modified only to give fine control and very steady rates of feed. As well as " Drikold ", liquid-air traps were used in the line after the reactor to assist in recovery of products.

Methods of Analysis.—(*a*) *Combined nitrogen.* The normal Kjeldahl method was used for the determination of recovery of total nitrogen and of nitrile, the ammonia being absorbed in saturated boric acid solution, and titrated with methyl-red as indicator (Mecker and Wagner, *Ind. Eng. Chem. Anal.*, 1933, **5**, 396). The accuracy was within 1—2%.

(*b*) *Pyridine, 2-methylpyridine, and 2-phenylpyridine.* The method of analysis used was a slight modification of that described by Ladd (*Ind. Eng. Chem.*, 1919, **11**, 522). The steam-distillate containing the pyridinic product was made up to an appropriate volume with a suitable amount of N/5-hydrochloric acid. Aliquots were titrated to (i) methyl-orange/methylene-blue, (ii) phenolphthalein. The difference in titrations calculated to the original volume gave the amount of pyridinic product. In the absence of large amounts of ammonia the method was sensitive to about 0·005 g. of pyridinic compound.

(*c*) *2-Cyanopyridine.* This was determined by hydrolysis by excess of 10% sulphuric acid to ammonia (2—3 hrs.' refluxing), which was estimated as above.

(*d*) *Cyanogen and hydrogen cyanide.* For cyanogen, Ricca's method of analysis was used (*Ann. Chim. appl.*, 1926, **16**, 83). The accuracy was about 1%. The hydrogen cyanide was determined by absorption in sodium hydroxide solution and titration of aliquot samples with silver nitrate as in the method for cyanogen.

Characterisation of Pyridinic Product.—The physical constants and analyses by means of which the pyridinic products were identified were as follows : (*a*) Pyridine; picrate, m. p. and mixed m. p. 164—165° (lit., 167°) (Found : C, 42·25; H, 2·3; N, 18·9. Calc. : C, 42·9; H, 2·6; N, 18·2%). (*b*) 2-Methylpyridine; picrate, m. p. and mixed m. p. 165—167° (lit., 167—169°) (Found : C, 44·8; H, 3·4; N, 17·3. Calc. : C, 44·7; H, 3·1; N, 17·4%). (*c*) 2-Phenylpyridine; b. p. 134—138°/10 mm., $n_D^{25°}$ 1·6170 (Found :

C, 84·1; H, 6·1; N, 9·4. Calc.: C, 85·1; H, 5·1; N, 9·0%); picrate, m. p. 168—170° (lit., 169—170°) (Found: C, 53·2; H, 3·2; N, 14·4. Calc.: C, 53·1; H, 3·15; N, 14·7%).

Results.—The data and results for each run are summarised in Table IV. The material recoveries and nitrogen balances, as well as the yields of pyridinic product calculated on the nitrile "conversion," are listed in Table V. These yields are undoubtedly low since the nitrile conversion includes any losses

TABLE IV.

Summary of experimental data and results.

Run.	T°, c.	Space velocity, l./hr./c.c.	Mol. ratio $\frac{R{\cdot}CN}{C_4H_6}$	C_4H_6 input rate, mol./hr.	Duration, hrs.	Pyridinic product (total), mols. × 10^{-4}.	C_4H_6 conversion into pyridinic product (mols. %).	Space-time yield of pyridinic product, mols. × 10^{-4}/hr./100 c.c.
(*a*) Butadiene and cyanogen. 2-Cyanopyridine.								
1	400	0·0874	2·5	0·060	7·0	533	12·8	—
2	400	0·307	3·0	0·047	5·0	11·5	0·49	16·7
(*b*) Butadiene and hydrogen cyanide. Pyridine.								
6	404	0·278	3·6	0·215	1·0	2·41	0·11	3·0
2	597	$0·036_2$	1·0	0·320	7·5	334	1·39	
3	595	$0·038_1$	1·1	0·320	5·0	181	1·14	
(*c*) Butadiene and acetonitrile. 2-Methylpyridine.								
8	395	0·342	2·8	0·056	5·0	1·30	$0·04_6$	1·88
11	400	$0·057_1$	3·1	0·051	5·0	1·40	$0·05_5$	
7A	599	$0·057_8$	$1·7_2$	0·250	4·0	96·8	0·97	
7B	597	0·108	10·1	0·176	2·0	27·0	0·74	
(*d*) Butadiene and benzonitrile. 2-Phenylpyridine.								
6	393	0·531	3·0	0·083	3·0	0·60	$0·02_4$	1·45
24*	400	0·182	3·3	0·094	2·75	2·49	$0·09_6$	1·80
4	400	$0·015_2$	2·7	0·073	4·8	2·66	$0·07_8$	
5	402	$0·004_7$	3·2	0·020	12·0	2·56	0·106	
3	495	$0·014_6$	3·3	0·061	5·0	7·75	$0·25_8$	
1	530	0·019	1·4	0·142	9·7	19·3	$0·15_2$	
2A	591	0·036	1·7	0·244	3·0	25·8	$0·35_2$	
2B	590	0·072	6·0	0·188	2·3	21·9	$0·51_5$	

* Reaction chamber packed with Pyrex-glass chips.

TABLE V.

Summary of data in total material and nitrile recoveries.

Run.	Material recovery (total), % of input.	Combined N recovery (total), % of input.	Nitrile "conversion," % of nitrile input.	Yield of pyridinic product, % on conversion.
(*a*) Butadiene and cyanogen.				
1	98·0	—	15·0	34·0
2	95·4	—	30·5	0·63
(*b*) Butadiene and hydrogen cyanide.				
6	100	—	3·2	0·93
2	94·0	—	26·0	6·0
3	79·0	—	18·5	5·4
(*c*) Butadiene and acetonitrile.				
8	89·2	91·0	18·2	0·1
11	90·0	90·5	18·0	0·1
7A	94·0	97·0	10·0	5·6
7B	97·5	97·0	7·0	1·0
(*d*) Butadiene and benzonitrile.				
6	96·5	100	5·5	$0·1_4$
24	100	96·5	4·5	$0·7_2$
4	95·5	101	4·0	$0·6_5$
5	93·0	98·5	5·0	$0·7_3$
3	97·5	99·1	2·0	3·0
1	93·5	96·0	4·0	2·6
2A	95·0	100	1·5	10—20
2B	97·0	96·5	2·5	1—3

incurred in the experimental recovery procedure which involved several operations, *i.e.*, distillations and ether extractions. The space velocity is expressed in l. of flow per hr. per c.c. of reactor volume, and the space-time yield as moles $\times 10^{-4}$ of pyridinic product per hr. per 100 c.c. of reactor volume. The conversion of butadiene into pyridinic product, calculated as % of the butadiene input, is a sufficient criterion of the extent of the reaction per pass.

The data in Table V show that with the exception of the benzonitrile–butadiene reaction, the nitrile conversions are all high per pass, and the resulting yield of pyridinic product correspondingly low. Some of this conversion is undoubtedly accounted for by side reactions, such as polymerisation (*e.g.*, to paracyanogen), decomposition (*e.g.*, to nitrogen and carbonaceous residues), and reduction to amines and ammonia (detected, but not estimated), but this aspect of the work was not followed.

The authors wish to thank Imperial Chemical Industries Limited for gifts of chemicals, the Chemical Society for a grant, the University of London for the award of an I.C.I. Fellowship to one of them (G. J. J.), and Professor C. K. Ingold, F.R.S., and Dr. Albert Wassermann for continued interest in this work.

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UNIVERSITY COLLEGE, LONDON, W.C. 1. [*Received, October 29th*, 1948.]

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316. *The Reaction of Cyanogen and Related Nitriles with* 1 : 3-*Dienes. Part II. Catalysis of the Reaction of Cyanogen and Related Nitriles with* 1 : 3-*Butadiene.*

By P. J. HAWKINS and G. J. JANZ.

The catalysis of the cyclisation reaction between organic nitriles and dienes to form pyridinic products is reported. Of the catalysts tested a mixture of chromium oxide and activated alumina was most promising. The activity–composition relationship of this catalyst was studied, as well as its activity in the cyclisation when only the nitrile was varied. The region of enhanced activity found indicates there is a promoter type of activity in this mixed catalyst. In the presence of the catalyst, the order of the space-time yields for these reactions has been reversed by comparison with that observed for the uncatalysed reactions, and the order now seems to be related to the polarisability of the organic nitrile.

IN a search for catalysts for the reactions described in the preceding paper, the preliminary activity measurements were made on the butadiene–benzonitrile reaction. The ratio of the space-time yield in the catalysed to that in the uncatalysed reaction, under the same conditions of space velocity, temperature, etc., was taken as the measure of the activity of the catalyst. The measurements were carried out at a relatively low temperature (400°) since the cyclisation is thermodynamically more favoured in this range, and at fairly rapid space velocities to minimize complications from secondary reactions. The catalysts investigated, and the activities found, in the preliminary search were as follows :

Catalyst.	Activity.
(Z–1), Activated alumina	5·08
(Z–2), 10% Nickel on alumina	3·76
(Z–3), Phosphoric acid on kieselguhr	1·91
(Z–4), Phosphoric acid on alumina	3·55
(Z–5*a*), 13% Chromium oxide on alumina	11·0
(Z–6), 10% Iron oxide on alumina	6·96
(Z–7*a*), Chromium oxide	1·91
(Z–5*b*), 5% Chromium oxide on alumina	17·8

The results indicate that catalysts favouring cyclisation and dehydrogenation (Taylor and Turkevich, *Trans. Faraday Soc.*, 1939, **35**, 921; Rideal and Herington, *Proc. Roy. Soc.*, 1945, *A*, **184**, 447; Steiner, *J. Inst. Petr.*, 1947, **33**, 410; Kline and Turkevich, *J. Amer. Chem. Soc.*, 1944, **66**, 1710) are the most active for this reaction. The mixed catalyst, chromium oxide–activated alumina, was chosen for further investigation in order to establish the relation between the enhanced activity and the composition of the catalyst.

The results of the activity–composition study are shown in Fig. 1 (see Table I*A*, Runs 6, 7, 25, 26, 22, 11, 12, 15, 16, 23, 18AB, 20, and 14). There is a region of enhanced activity for mixtures containing relatively small amounts of chromium oxide. The values for the 13% chromium oxide catalyst were obtained in tests where the space velocity was varied, and within the limits investigated, the activity is independent of such variations. The two results for the 100% chromium oxide catalyst were obtained with oxide prepared by different methods. The smaller of the two values found for the 45% chromium oxide catalyst was obtained when the catalyst was not exposed to the standard pre-treatment with hydrogen. The nature of the

curve is indicative of a promoter action in the mixed catalyst, rather than simple compound formation, or mechanical mixture behaviour (Griffith, " Contact Catalysis," Oxford University

FIG. 1.

Activity–composition relationship for the mixed catalyst, chromium oxide–activated alumina in the benzonitrile–butadiene cyclisation at 400° C.

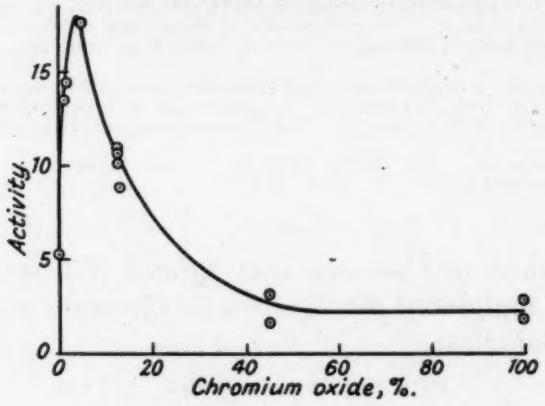


TABLE I.

Summary of data and results.

Run.	Catalyst.	T (°C.).	Space velocity, l./hr./c.c.	Ratio (mol.) $\frac{R{\cdot}CN}{C_4H_6}$.	C_4H_6 input (mols./hr.).	Pyridinic product (mols. $\times 10^{-4}$).	C_4H_6 (mols. %) conversion to pyridinic product.	Space-time yield mols. $\times 10^{-4}$/hr./100 c.c. catalyst).	Activity of catalyst.	Duration of test (hrs.).
(*A*) Benzonitrile and butadiene: 2-phenylpyridine.										
6	—	393	0·531	3·0	0·083	0·60	$0·02_4$	1·45	—	3·0
7	Z–1	396	0·525	2·5	0·093	3·09	$0·11_2$	7·38	5·08	3·0
10	Z–2	398	0·272	2·3	0·048	2·12	$0·14_7$	5·45	3·76	6·0
13	Z–3	398	0·418	3·3	0·056	1·10	$0·06_3$	2·77	1·91	3·0
8	Z–4	396	0·482	2·8	0·086	2·32	$0·09_0$	5·16	3·55	3·0
11	Z–5*a*	397	0·288	2·4	0·047	12·0	$0·42_6$	16·0	11·0	6·0
16	Z–5*a*	402	0·049	3·0	0·160	167	2·85	15·7	10·8	3·66
21	Z–6	400	0·241	4·7	0·120	12·3	0·53	10·1	6·96	1·92
14	Z–7*a*	393	0·323	3·2	0·048	1·17	0·81	2·77	1·91	3·0
22	Z–5*b*	400	0·234	2·9	0·107	30·9	$0·96_3$	25·8	17·8	3·0
25	Z–5*c*	400	0·265	3·1	0·074	15·5	$0·69_6$	20·1	13·8	3·0
26	Z–5*d*	400	0·265	2·6	0·106	20·1	$0·65_4$	21·0	14·5	3·0
12	Z–5*a*	400	0·055	3·2	0·046	34·8	2·53	14·9	10·3	3·0
15	Z–5*a*	398	0·307	2·6	0·054	5·22	$0·33_4$	12·9	8·90	3·0
23	Z–5*e*	396	0·311	2·3	0·112	4·68	0·14	5·12	3·53	3·0
18AB	Z–5*e*	398	0·262	3·3	0·149	4·50	0·10	2·64	1·82	3·0
20	Z–7*b*	400	0·538	4·1	0·108	3·09	$0·09_6$	4·52	3·11	2·0
17AB	Z–5*a*	475	0·233	3·1	0·189	135	2·50	6·29	43·4	2·75
19AB	Z–5*e*	475	0·255	3·2	0·151	12·4	0·36	9·68	6·68	2·75
(*B*) Acetonitrile and butadiene: 2-methylpyridine.										
8	—	395	0·342	2·8	0·056	1·30	$0·04_6$	1·88	—	5·0
12	Z–1	400	0·264	2·9	0·043	7·00	0·32	10·0	5·32	5·0
13	Z–5*a*	398	0·286	3·2	0·038	5·85	0·51	15·6	8·29	3·0
15	Z–1	301	0·062	2·5	0·051	1·62	0·10	0·63	0·34	3·0
14	Z–5*a*	300	0·062	3·6	0·052	1·62	0·10	0·63	0·34	3·0
16AB	Z–5*a*	475	0·346	3·0	0·176	115	0·98	38·2	20·31	3·0
9	Z–1	398	0·048	3·2	0·043	27·0	1·26	6·30	3·55	5·0
(*C*) Hydrogen cyanide and butadiene: pyridine.										
6	—	404	0·278	3·6	0·215	2·41	0·11	3·0	—	1·0
7	Z–5*a*	404	0·284	3·1	0·096	9·78	0·37	11·4	3·80	2·75
(*D*) Cyanogen and butadiene: 2-cyanopyridine.										
2	—	400	0·307	3·0	0·047	11·5	0·49	16·7	—	5·0
3	Z–5*a*	400	0·253	3·0	0·040	62·3	5·22	149	8·92	3·0

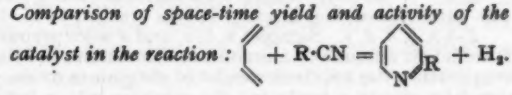
TABLE II.

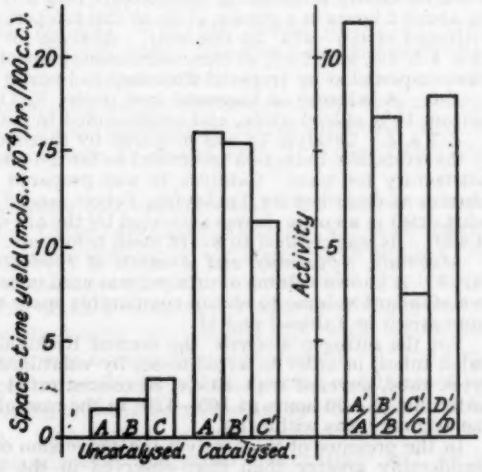
Summary of material and nitrile recoveries.

Run.	Catalyst.	*T* (° c.).	Material recovery (total), % of input.	Combined-nitrogen recovery (total), %.	Conversion of nitrile, % of input.	Pyridinic product yield, calc. on nitrile converted, %.
(*A*) Benzonitrile and butadiene: 2-phenylpyridine.						
22	Z–5*b*	400	99·0	98·7	4·3	8·0
11	Z–5*a*	400	97·2	100	3·7	4·8
17	Z–5*a*	475	96·0	97·2	5·5	14·1
(*B*) Acetonitrile and butadiene: 2-methylpyridine.						
13	Z–5*a*	400	95·5	93·5	19·5	0·83
(*C*) Hydrogen cyanide and butadiene: pyridine.						
7	Z–5*a*	400	80·3	78·0	22·7	0·53
(*D*) Cyanogen and butadiene: 2-cyanopyridine.						
3	Z–5*a*	400	81·3	—	22·3	8·5

Press, 1948). It is significant that Eischens and Selwood (*J. Amer. Chem. Soc.*, 1947, **69**, 1590) found that the magnetic susceptibility of chromium oxide on alumina decreases with increasing concentration of chromium oxide, but in the region of low chromium oxide compositions it is much larger than for the higher concentrations. The region of low chromium oxide compositions is the region where the enhanced catalytic activity was observed, and it would seem that the activated alumina serves the two-fold purpose of maintaining and increasing the extent of the chromium oxide surface and of providing a surface of chromium oxide of proper nature for catalytic activity. Both the reactants are unsaturated and are probably sensitive to the nature of the metallic oxide surface. As to the actual mode of action of these catalysts there is very little information. Rideal and Herington (*loc. cit.*) in cyclisation studies using a MoO_2-activated alumina catalyst and hydrocarbons, attributed the activity of the MoO_2 to the presence of two unpaired electrons in the ion.

FIG. 2.

Comparison of space-time yield and activity of the catalyst in the reaction : 



In A, A' . . ., R = C_6H_5; *in B, B' . . .*, R = CH_3; *in C, C' . . .*, R = H; *in D, D' . . .*, R = CN.

In Fig. 2 a comparison is made of the results for the uncatalysed and the catalysed reaction when cyanogen, hydrogen cyanide, acetonitrile, and benzonitrile are used in turn with butadiene (see Table I*A*, 6, 11; Table I*B*, 8, 13; Table I*C*, 6, 7; Table I*D*, 2, 3). On comparison with the results of the uncatalysed study, it is seen that the space-time yield has in each case been markedly increased, and also that the trend, except with cyanogen, is now the reverse of that formerly observed. Considering the activity of the catalyst, one finds that it decreases in the order R = phenyl, methyl, hydrogen. Although the phenomenon undoubtedly involves other factors as well, it would seem that the polarisation of the nitrile group at the catalyst surface is an important factor in the mechanism of the reaction, for the above sequence is also the order in which the polarizability of the cyano-group varies for these nitriles, *i.e.*, greatest for benzonitrile and least for hydrogen cyanide. The order of the catalytic effect in the cyanogen–butadiene reaction is difficult to interpret because there are two potential reaction centres per molecule of nitrile. It is seen, however, that it lies below the value for the benzonitrile–butadiene reaction, and this is in accord with the above discussion. Studies of this cyclisation with dienes other than butadiene are in progress in order to obtain further information on this question.

The effect of temperature on the rate of the catalysed reaction is seen on comparison of some of the data in Table I (*i.e.*, I*A*, 11, 17AB, 18AB, 19AB, I*B*, 15, 12, 14, 13, 16AB). When using activated alumina only, or the chromium oxide-activated alumina mixture, it was found that the reaction occurred appreciably at temperatures as low as 300°, and that the rate was markedly accelerated at 475°. It should be noted that the activity figures at these temperatures are only qualitative since they have been calculated with the uncatalysed results at 400° as the standard of reference. At 300°, catalytic activity being judged this way, it is seen that the mixed catalyst has the same activity as the alumina by itself.

The poisoning of the catalysts has not been studied, but their activity decreased with reaction time, more so at the higher temperatures than at 400°. For this reason the duration of the runs in the activity studies was standardised to about 3 hours. The catalysts could be revived by oxidation and hydrogen pre-treatment in the usual manner.

Experimental.

Preparation of Catalysts.—*Z*-1. A commercial grade of activated alumina (Messrs. Peter Spence & Sons Ltd.), 8—16 mesh, was used. A slow stream of air was passed over the catalyst at the reaction temperature, followed by a nitrogen flush, before each test.

Z-2. The catalyst was prepared essentially as described by Kline and Turkevich (*loc. cit.*). A solution of nickel nitrate, sufficient to give about 10% of nickel, was evaporated on the alumina. The nitrate was then decomposed at 300—400°, and the mixture heated in air at 300°. The catalyst was finally flushed with nitrogen and reduced with hydrogen, also at 300°, for approximately one hour.

Z-3. A paste of phosphoric acid (306 g.; *d* 1·75) and kieselguhr (108 g.) was heated at about 450—500° until a cement had formed. This was powdered to 8—16 mesh for the tests.

Z-4. A mixture of activated alumina (100 g.) and phosphoric acid (100 g.; *d* 1·75) was heated at 400—450° for several hours. It was crushed to 8—16 mesh before use.

Z-5 *a*, *b*, *c*, *d*, *e*. Samples *a*, *b*, *c*, and *d* were prepared essentially as described by Kline and Turkevich. A sufficient amount of ammonium dichromate in solution was mixed with activated alumina to give on evaporation the required weight of chromium oxide, calculated as Cr_2O_3. The dichromate was decomposed by slowly introducing the mixture into a furnace at 400—475°. The catalyst was then heated for about 2 hours in a stream of air at this temperature. It was flushed with nitrogen and reduced with hydrogen at 450—475° for one hour. Analysis for chromium gave the compositions for *a*, *b*, *c*, and *d* as 12·8, 4·5, 1·0, and 2·0% of chromium oxide respectively. Catalyst 5*e* containing 45% chromium oxide was compounded by Imperial Chemical Industries Limited.

Z-6. A mixture of magnetic iron oxide, Fe_3O_4 (B.D.H.), and activated alumina was prepared to contain 10% of iron oxide, and compounded to pellets suitable for the activity tests.

Z-7 *a*, *b*. Catalyst 7*a* was prepared by thermal decomposition of ammonium dichromate, followed by the oxidation–reduction treatment as for the mixed catalysts. It was difficult to pellet and not very satisfactory for tests. Catalyst 7*b* was prepared by reduction of chromic acid in aqueous-alcoholic solution as described by Turkevich, Fehrer, and Taylor (*J. Amer. Chem. Soc.*, 1941, **63**, 1129). After being dried in an oven, it was activated by the air–nitrogen–hydrogen treatment as for the Z-5 catalysts, at 450°. It was crushed to 8—16 mesh before use.

Materials, Apparatus, and Analysis of Products.—These were essentially the same as described in Part I. A known volume of catalyst was used in each case, and the results were subsequently calculated to a standard volume to obtain comparable space-time yields and activities. The data and results are summarised in Tables I and II.

For the nitrogen analysis, the normal Kjeldahl digestion was abandoned in favour of digestion in sealed tubes, in order to avoid losses by volatilisation. About 0·5 g. of the sample was weighed in a Pyrex tube, covered with 10 c.c. of concentrated sulphuric acid and sealed off. The tube was then heated for 15—20 hours at 100—120° in the case of acetonitrile, and 150—175° in that of benzonitrile. The accuracy was within 1%.

In the presence of the catalyst the conversion of the nitrile into ammonia and related products was considerably greater than that observed in the uncatalysed reactions. The material and nitrogen balances listed in Table II are typical of the results found. The nitrile conversions as listed here include also the nitrile lost in experimental procedure, and the yields calculated on these conversions can therefore be improved if these losses can be further decreased. The total material recovery figures indicate that some of the loss is incurred over the catalyst bed, owing probably to carbonaceous products being formed and deposited on the bed, and to pyrolysis to gaseous products such as methane, ethylene, and nitrogen. This aspect of the work has not yet been followed further.

The authors wish to thank Professors E. D. Hughes and C. K. Ingold for continued interest in this investigation, and Imperial Chemical Industries Limited, and the Chemical Society for gifts of, and a grant for, special chemicals, and the University of London for an I.C.I. Fellowship granted to one of them (G. J. J.).

The Sir William Ramsay and Ralph Forster Laboratories,
University College, London, W.C. 1. [*Received, October, 29th,* 1948.]

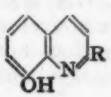
317. *Steric Hindrance in Analytical Chemistry. Part I.*

By Harry Irving, E. J. Butler, and M. F. Ring.

8-Hydroxy-2-, -5-, and -6-methylquinoline, 8-hydroxy-1-phenylquinoline, 1-hydroxyacridine, and 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* have been prepared as potential precipitating reagents in metal analysis and found to have much the same sensitivity as 8-hydroxyquinoline ("oxine"), whilst that of 8-hydroxy-7-methylquinoline is somewhat greater. All gave insoluble complexes with Cr^{+++}, Fe^{+++}, Ga^{+++}, Cu^{++}, and Zn^{++}, but 8-hydroxy-2-methyl-, -2 : 4-dimethyl-, and -2-phenyl-quinoline, 1-hydroxyacridine, and 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine differed from the others in failing to give an insoluble complex with Al^{+++}. A possible explanation in terms of the stereochemistry of these complexes is advanced, and its implications are considered in connexion with the behaviour of various charged and uncharged complexes of analytical importance. The efficiency as bacteriostatic agents of several of these derivatives and analogues of oxine has been measured and discussed in terms of a contemporary theory of drug action.

It is well known that aluminium forms a stable complex of stoicheiometric composition with 8-hydroxyquinoline ["oxine" (I; R = H)] which permits of its determination by gravimetric, volumetric, and absorptiometric procedures (Berg, "Die Analytische Verwendung von *o*-Oxychinolin," Stuttgart, 1938; Sherrington and Gentry, *Analyst*, 1946, **71**, 433). Since very many other elements behave similarly, lack of selectivity is an adverse feature in the use of oxine necessitating careful adjustment of pH and choice of masking agents when the constituents of certain mixtures are to be determined. It is often difficult and sometimes impossible to avoid co-precipitation of metal–oxine derivatives [Chirnside, Pritchard, and Rooksby, *Analyst*, 1941, **66**, 399; Moyer and Remington, *Ind. Eng. Chem.* (*Anal.*), 1938, **10**, 212]. That the introduction of substituents might produce a reagent more sensitive or selective was first considered by Berg (*Z. anorg. Chem.*, 1932, **204**, 208), and 5 : 7-dibromo-8-hydroxyquinoline was developed as a special reagent for copper, iron, and titanium (Berg and Küstenmacher, *ibid.*, p. 215). A number of 5-arylazo-derivatives of oxine were examined by Gutzeit and Monnier (*Helv. Chim. Acta*, 1933, **16**, 233, 478), and many others by Boyd, Degering, and Shreve [*Ind. Eng. Chem.* (*Anal.*), 1938, **10**, 606]; like the 7-arylazo-derivatives of 8-hydroxy-5-methylquinoline (Irving and Taylor, unpublished), they are more sensitive than oxine itself, and several of them may be recommended as selective reagents for palladium, mercury, vanadium, and, possibly, copper. 7-Iodo-8-hydroxyquinoline-5-sulphonic acid has been carefully studied as a colorimetric reagent for iron (Yoe and Hill, *J. Amer. Chem. Soc.*, 1937, **59**, 872).

Now, whilst 8-hydroxy-5-methylquinoline appears to resemble oxine very closely so far as it has yet been studied (Gietz and Sa, *Anal. Asoc. Quím. Argentina*, 1935, **23**, 45), 8-hydroxy-2-methylquinoline (I; R = CH_3) displays a remarkable difference, for Merritt and Walker [*Ind. Eng. Chem.* (*Anal.*), 1944, **16**, 387] found that it would not give a precipitate with aluminium, a peculiarity which they exploited by introducing it as a reagent for zinc in the presence of aluminium and magnesium. Comparing 2-methyloxine (I; R = CH_3) with oxine they remark: "Probably because of its increased size, it is a more selective reagent. If the size of the molecule is the determining factor, the larger molecule might be expected not to react with the smaller ions because of the difficulty of grouping three large molecules around the small ion. If a complex is formed it might be less stable." No further communication has appeared in this field and, since the observation is of considerable analytical importance and no less interesting academically, we have considered its implications in some detail. To this end we prepared 8-hydroxy-2-, -5-, -6-, and -7-methyl-, -2 : 4-dimethyl-, and -2-phenyl-quinoline, 1-hydroxyacridine (II), and 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III), and compared the sensitivity of their reactions with those of oxine towards aluminium and a number of other metals.



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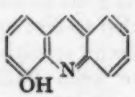

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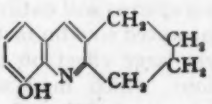


(III.)

Preparation of Reagents.—8-Hydroxy-2-, -6-, and -7-methylquinolines were prepared by modifications of published syntheses (cf. Merritt and Walker, *loc. cit.*; Herzfeld, *Ber.*, 1884, **17**, 905, 1552; Fischer, *ibid.*, p. 441; Noelting and Trautmann, *Ber.*, 1890, **23**, 3663). Although *o*-aminophenol and acetylacetone condensed smoothly to give 4-(*o-hydroxyanilo*)*pentan-2-one*,

o-OH·C_6H_4·N:CMe·CH_2·COMe, this anil resisted all attempts at ring-closure to form 8-hydroxy-2 : 4-dimethylquinoline. However, aniline and acetylacetone readily condensed to give 2 : 4-dimethylquinoline (Scheibe, Merkel, and Müller, *J. pr. Chem.*, 1919, **100**, 91), which was readily sulphonated, but gave only a very poor yield of the desired hydroxyquinoline on alkali fusion. 8-Hydroxy-2-phenylquinoline, prepared by decarboxylation of 8-hydroxy-2-phenylquinoline-4-carboxylic acid, formed almost colourless needles, m. p. 85·5° (Doebner and Fettbach, *Annalen*, 1894, **281**, 9, give m. p. 59°). A more direct synthesis by the action of phenyl-lithium upon oxine gave plates m. p. 58—59°. Both preparations give satisfactory analytical figures for $C_{15}H_{11}ON$ and are probably polymorphs; the picrate from each melted at 159° and did not depress the melting point of the other.

1-Hydroxyacridine has already been synthesised by several routes (cf. Jensen and Rethwisch, *J. Amer. Chem. Soc.*, 1928, **50**, 1144; Albert and Ritchie, *J.*, 1943, 458). On heating 2-ethoxydiphenylamine-2′-carboxylic acid with concentrated sulphuric acid, Matsumara (*J. Amer. Chem. Soc.*, 1927, **49**, 811) obtained a mixture of 1-hydroxy- and 1-ethoxy-acridone, from which the former was separated and then reduced by sodium in amyl alcohol to 1-hydroxyacridine. Starting from 2-methoxydiphenylamine-2′-carboxylic acid, Freeman and Lions (*Proc. Roy. Soc. N.S. Wales*, 1940, **74**, 520) found difficulty in repeating this preparation, but claimed a 60% yield of hydroxyacridine by a modified process which they did not describe in detail. Ullmann (*Annalen*, 1907, **355**, 342) believed the yellow substance, m. p. 293°, which he obtained by the action of concentrated sulphuric acid on 2-methoxydiphenylamine-2′-carboxylic acid to be 1-methoxyacridone. In our hands this yellow product of ring-closure always contained sulphur and gave analytical figures approaching those for a monosulphonated methoxyacridone. Variation in the concentration of acid, temperature, and duration of heating, and the use of acetic–sulphuric acid mixtures failed to give sulphur-free products (cf. Matsumara, *J. Amer. Chem. Soc.*, 1935, **57**, 1533). Authentic 1-*methoxyacridone* was, however, obtained as buff-coloured needles, m. p. 284—285° by heating 2-methoxydiphenylamine-2′-carboxylic acid with phosphorus oxychloride and hydrolysing the intermediate 5-chloro-1-methoxyacridine with boiling dilute hydrochloric acid (cf. Albert and Linnell, *J.*, 1936, 1614). Though reduction with sodium amalgam (Bradbury and Linnell, *J.*, 1942, 377) did not prove satisfactory, the product obtained by reducing 1-methoxyacridone with sodium and amyl alcohol (Kranzlein, *Ber.*, 1937, **70**, 1785) was oxidised smoothly by silver nitrate to 1-methoxyacridine, identical with an authentic specimen. Demethylation with fuming hydrobromic acid (cf. King and Sherred, *J.*, 1942, 415) gave 1-hydroxyacridine (II) in 50% overall yield. 9-Methoxy-1 : 2 : 3 : 4-tetrahydroacridine, prepared by a modification of Petrow's method (*J.*, 1942, 693), was similarly demethylated to 9-*hydroxy*-1 : 2 : 3 : 4-*tetrahydroacridine* (III).

Testing of Reagents.—Following Lutz (*Z. anal. Chem.*, 1920, **59**, 145), it has become the practice to record the sensitivity of a reagent in terms of the smallest amount of metal which will give a perceptible precipitate in a fixed volume of a test solution under standardised conditions. Berg (*loc. cit.*) used three test solutions containing respectively (i) 1 ml. of saturated sodium acetate and 0·5 ml. of 2N-acetic acid, (ii) 1 ml. of 2N-ammonia, and (iii) 0·5 ml. of saturated sodium tartrate and 0·5 ml. of 2N-sodium hydroxide, in a total volume of 5 ml. When the reactions of kations precipitable by ammonia were examined, a few drops of saturated sodium tartrate were added to solution (ii). In each test a few drops of a 2% alcoholic solution of the organic reagent were added.

Now a complex MR_n will not be precipitated until $[M^{n+}][R']^n$ equals or exceeds S_{MR_n}, the solubility product. The observed sensitivity will thus be influenced by the " visibility " of the precipitate and will depend on the concentration of reagent, HR, employed and especially upon the pH of the solution, since the pH will determine what fraction of the reagent is present as the participating ion, R′, and what fraction of the metal added is present as the (hydrated) kation, M^{n+} — a factor of importance if it shows amphoteric character. Masking reagents which form competitive complexes will naturally reduce the measured sensitivity, and small variations in the amount of saturated sodium tartrate added to test solutions (see above) may thus produce a disproportionately large effect on the apparent sensitivity. Berg does not record the pH of his test solutions, which may well have been liable to variation unless the volume measurements were reproduced with some accuracy.

In the present work the three test solutions of pH 5·2, 8·4, and 12·4, respectively, were prepared in bulk from known weights of pure reagents. In each test the total volume was identical, *viz.*, 6·2 ml., and each organic reagent was then present in 0·00323M. concentration. Table I records for each combination the smallest concentration (in μg./ml.) of metal which gave a perceptible precipitate, together with the largest concentration which just failed to do so

TABLE I.

Metal used :	Al^{+++}.			Cr^{+++}.			Fe^{+++}.			Cu^{++}.			Zn^{++}.			Ga^{+++}.		
Test solution :	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*	*A.*	*B.*	*C.*
8-Hydroxyquinoline.	4·4— 1·7 (yellow)	4·4— 1·7	N.P.	8·4— 3·4 (yellow-brown)	16·8— 8·4	N.P.	1·8— 0·9 (greenish-black)	1·8— 0·9	N.P.	1·0— 0·4 (yellow-green)	1·0— 0·4	1·0— 0·4	1·1— 0·4 (yellow)	1·0— 0·4	10·6— 4·2	2·3— 1·1 (yellow)	2·3— 1·1	N.P.
8-Hydroxy-2-methylquinoline.	N.P.	N.P. (—)	N.P.	3·4— 1·7 (yellow-brown)	1·7— 0·8	N.P.	1·8— 0·9 (greenish-black)	0·9— 0·4	N.P.	1·0— 0·4 (salmon)	1·0— 0·4	20·5— 10·3	21·0— 10·6 (yellow)	1·0— 0·4	2·1— 10·6	22·5— 11·3 (yellow)	2·3— 1·1	N.P.
8-Hydroxy-5-methylquinoline.	0·9— 0·4 (lemon-yellow)	*	N.P.	1·7— 0·8 (orange)	*	N.P.	3·64 1·8 (greenish-black)	0·9— 0·4 *	N.P.	1·0— 0·4 (yellow)	*	1·0— 0·4	10·6— 4·2 (yellow)	*	N.P.*	2·3— 1·1 (yellow)	*	N.P.
8-Hydroxy-6-methylquinoline.	0·4— 0·2 (yellow)	(—)	N.P.	3·4— 1·7 (orange)	—	N.P.	0·9— 0·36 (dark green)	1·8— 0·9 §	N.P.	1·0— 0·4 (yellow)	—	2·1— 1·0	2·1— 1·1	10·6— 2·1 §	10·6— 4·2 §	1·1— 0·45	2·3— 1·1 §	N.P.
8-Hydroxy-7-methylquinoline.	0·4— 0·09 § (lemon-yellow)	0·44— 0·17 §	N.P.§	0·34— 0·17 §	0·8— 0·34 §	N.P.	0·9— 0·36 § (greenish-black)	0·9— 0·36 §	36— 18 §	0·1— 0·04 §	1·0— 0·4 § (yellow)	2·1— 1·0 §	21·0— 10·6 §	4·2— 2·1 § (yellow)	2·1— 1·1 §	1·1— 0·23 §	1·1— 0·45 § (yellow)	22·5— 11·3 §
8-Hydroxy-2-phenylquinoline.	N.P.	N.P. (—)	N.P.	0·84— 0·34 * (yellow-brown)	34— 17 *	N.P.	0·9— 0·36 * (yellow-brown)	0·9— 0·36 *	N.P.	1·0— 0·4 * (yellow)	1·0— 0·4 *	2·0— 1·0 *	10·6— 4·2 *	2·1— 1·1 * (yellow)	4·2— 2·1	1·1— 0·45 *	11·0— 4·5 * (yellow)	N.P.
1-Hydroxyacridine.	N.P.§	N.P.§ (—)	N.P.§	0·84— 0·34 §	8·4— 3·4 § (red)	N.P.§	1·8— 0·9 § (purplish-brown)	0·9— 0·36 §	N.P.§	2·1— 1·0 §	1·0— 0·4 § (red)	4·1— 2·0 §	21·1— 10·6 §	1·1— 0·42 § (red)	N.P.§	1·1— 0·45 §	0·45— 0·23 § (orange red)	N.P.§
9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine.	N.P.	N.P.§ (—)	N.P.	0·84— 0·34 (yellow-brown)	0·84— 0·34 §	N.P.	0·9— 0·36 (dark green-brown)	0·9— 0·36 §	N.P.	1·0— 0·41 (brown)	1·0— 0·41 §	2·1— 1·0	42·2— 21·1 (yellow)	1·0— 0·41 §	211— 105	4·5— 2·3 (pale yellow)	1·1— 0·45 §	N.P.

N.P. signifies that there was no precipitate of complex.

— signifies that no observation was made.

§ This symbol denotes observations made on a hot solution in those cases in which the organic reagent was insoluble in the cold test solution but soluble at 80°.

* Where the reagent was insoluble in both hot and cold solutions evidence of complex formation could sometimes be obtained if its colour or appearance was sufficiently distinctive.

Sensitivity limits (cf. p. 1490) are given as μg./ml.

The colour of the precipitated complex is recorded below the sensitivity limits.

8-Hydroxy-2 : 4-dimethylquinoline was tested only with aluminium and failed to form a complex in any of the three test solutions.

under the standardised conditions of testing detailed below (p. 1497). These seldom differed by a factor greater than two, and the larger value was adopted as a conservative estimate of sensitivity since more precise values were not required for the present work and, indeed, may well be without real significance (cf. Feigl, *Z. angew. Chemie*, 1930, **43**, 550). One expected result should be recorded. When oxine was tested with aluminium in the ammoniacal buffer (*B*), precipitation failed with concentrations of metal less than 4·4 μg./ml., but with 175 μg./ml. very little precipitate appeared, and with 220 μg./ml. or stronger aluminium solutions no precipitate at all was obtained. Calculation showed that in these concentrated solutions there was insufficient oxine to form the normal 3 : 1 complex, $AlOx_3$ (Ox = C_9H_6ON), and the metal is probably held in solution (as salt of the ions, $[Al(H_2O)_2Ox_2]^+$, $[Al(H_2O)_4Ox]^{++}$, or $[Al(OH)_2Ox_2]^-$, etc.

Discussion.—In agreement with Merritt and Walker, we find that 2-methyloxine (I) will not give a precipitate with aluminium under any conditions. However, since this peculiarity is not shared by any of the other methyloxines, the effect cannot be due simply to a general increase in size as they suggest. Since 2 : 4-dimethyloxine, 2-phenyloxine (I; R = C_6H_5), 1-hydroxyacridine (II), and 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine (III) likewise fail to form insoluble complexes with aluminium, whilst forming insoluble complexes with a number of other bi- and ter-valent kations, it must be concluded that this specific effect is caused by substitution adjacent to the nitrogen atom.

Now it has been shown (Goto, *J. Chem. Soc. Japan*, 1933, **54**, 725; 1935, **56**, 314; Fleck and Ward, *Analyst*, 1933, **58**, 388; 1937, **62**, 378) that, above a pH value characteristic for each element, the percentage of a metal precipitated by oxine rises rapidly from zero to its maximum value as the alkalinity increases. Over a range of hydrogen-ion concentrations, precipitation is complete, but the fraction precipitated rapidly decreases again to zero at high alkalinities with metals of amphoteric character. Similar characteristics are displayed by other organic precipitants. Table II records a sensitivity exponent p*L*, defined as p*L* = $-\log_{10}$ (limiting

TABLE II.

	Al^{+++}.	Cr^{+++}.	Fe^{+++}.	Cu^{++}.	Zn^{++}.	Ga^{+++}.	Average p*L* values with that for aluminium excluded.	Average p*L* values with that for aluminium included.
Oxine	3·3	3·3	4·0	4·5	4·5	4·0	4·1	3·9
2-Methyloxine	N.P.	4·0	4·3	4·5	4·5	4·0	4·3	—
5-Methyloxine	4·0	4·0	4·3	4·5	3·5	4·0	4·1	4·1
6-Methyloxine	4·3	3·7	4·3	4·5	4·2	4·3	4·2	4·2
7-Methyloxine	4·3	4·7	4·3	5·3	4·2	4·3	4·6	4·5
2-Phenyloxine	N.P.	4·3	4·3	4·5	4·2	4·3	4·4	—
1-Hydroxyacridine	N.P.	4·5	4·5	4·5	4·5	4·9	4·6	—
9-Hydroxytetrahydroacridine	N.P.	4·5	4·5	4·5	4·5	4·8	4·6	—

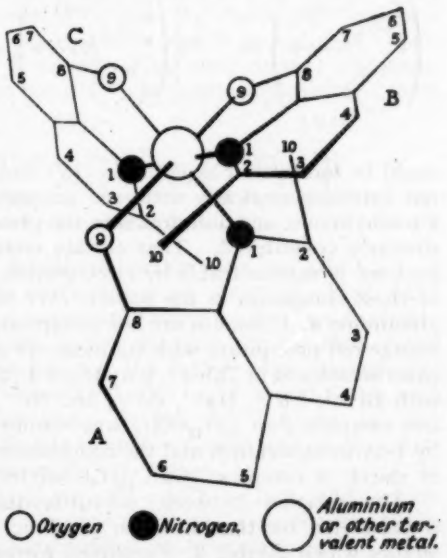
Values for oxine and derivatives cannot be compared directly with those for the acridines as these form 2 : 1 complexes. The composition of the complexes formed by 2-phenyloxine has not been determined.

concentration in gram-equivalents/l.). (In default of comprehensive data relating the percentage of element precipitated to the pH of the solution for each metal and reagent studied, and where the sensitivities in the three test-solutions *A*, *B*, and *C* were not identical, we have adopted values from the medium where the greatest sensitivity was shown.) When sensitivity is expressed in the customary manner as μg./ml., a false impression may be conveyed of the difference in sensitivities of the reactions of the same reagent with various metals of widely differing atomic weights or of different valency. This is to some extent corrected by the above device which, moreover, facilitates the comparison of one reagent with another.

It is perhaps surprising to note from Table II how small are the individual variations of sensitivity among the oxine derivatives from the average value, p*L* = 4·2. The most striking result is the superior average sensitivity of 7-methyloxine which we attribute to a steric effect discussed below (page 1495). The introduction of a methyl group elsewhere in the oxine skeleton produces only a slight general increase in sensitivity which, like that shown by 2-phenyloxine and the acridine derivatives (II) and (III) may well be related to the increased molecular weight and decreased solubility of these reagents, though the validity of such generalisations must await studies with a wider range of metals and an exact knowledge of the compositions of the complexes actually precipitated.

Whilst the oxine complexes of bivalent elements are often hydrated, those of tervalent

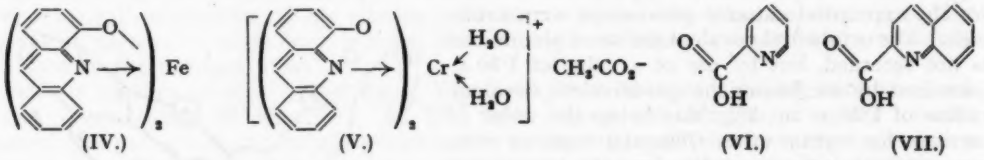
aluminium, iron, gallium, and indium are invariably anhydrous. *Gallium* and *indium* derivatives of 8-hydroxy-2-methylquinoline have now been prepared and, like the *thallic* derivative of 5 : 7-dibromo-8-hydroxyquinoline, these yellow complexes are also anhydrous and conform to the general formula, MR_3; they are all soluble in chloroform. Though Chirnside (*loc. cit.*) has used *X*-ray powder photographs in studying the co-precipitation of metal–oxine complexes, no complete determination of structure has yet been carried out, but it seems reasonable to suppose that with tervalent elements of co-ordination number six the three heterocyclic ligands form chelate rings in planes mutually at right angles. Apart from mirror-image isomerism two arrangements are possible. The figure depicts one of these possible arrangements for the 2-methyloxine complex of a tervalent metal. In the alternative configuration the three nitrogen atoms are located in the same quadrant diametrically opposite to the three oxygen atoms; but, since this arrangement would bring all three methyl groups into one and the same quadrant, the less crowded arrangement of the figure is more probable on energetic grounds. It will be noted that the 2-methyl group of each ligand is directed towards the oxygen (or nitrogen) atom of the neighbouring chelate ring at right-angles to it. The actual separation could be estimated by projections to scale in the planes of A, B, and C if reliable data for the appropriate atomic parameters were available. The octahedral covalent radius of aluminium is not recorded, but by use of a value of 1·30 A. (obtained by increasing the quadrivalent covalent radius of 1·26 A. by 3%, this being the order of increase for certain other elements) together with conventional atomic radii for the remaining elements the lengths C_{methyl}–Oxygen (B_{10} to A_9, and A_{10} to B_9) and C_{methyl}–Nitrogen (C_{10} to A_1) are nearly equal at about 2·7 A., *i.e.*, shorter than the distance of approach accepted for non-bonded atoms. The hydrogen atoms of the methyl group are naturally still closer, and it would appear that purely steric considerations would prohibit the formation of the aluminium complex of 2-methyloxine. However, judging from electronegativity differences (Pauling), the Al–O and Al–N bonds show some 65% and 45% ionic character. Magnetic data certainly show that the nickel complex of oxine contains ionic bonds ($\mu = 3{\cdot}4$ Bohr magnetons; Mellor and Craig, *Proc. Roy. Soc. N.S. Wales*, 1940, **74**, 479). Great uncertainty must therefore attach to the choice of bond angles and distances appropriate to the central chelate rings of complexes such as those depicted in the figure. Reference to a model shows how markedly any variation in these parameters may influence possible steric hindrance to complex formation, and, though simultaneous changes in the dimensions of the ring systems of the reagent may well be of subsidiary importance, the possibility of in-plane or out-of-plane displacement of the interfering 2-substituent cannot be ignored.



Whilst the ionic radius of aluminium is 0·50 A., that of gallium (which is reported as having the same tetrahedral covalent radius as aluminium, *viz.*, 1·26 A.), tervalent iron, chromium, indium, and thallium increase in the order 0·60, 0·62, 0·64, 0·81 and 0·95 A. The fact that we have found both gallium and indium to form well-defined 3 : 1 *complexes* with 2-methyloxine must not, however, be taken as evidence that mere increase of size has alone sufficed to reduce steric hindrance to negligible proportions. All these kations are less electropositive than aluminium and their bonds to oxygen and nitrogen should be more covalent in character; in certain cases (*i.e.*, with iron and chromium) additional orbitals are available which should lead to stronger bonds. The distinctive behaviour of aluminium in *not* forming complexes with certain derivatives of oxine substituted adjacent to the nitrogen atom is thus to be attributed to its (normally) small tendency towards such complex formation being still further reduced by the additional energy barrier imposed by unfavourable steric factors, a hypothesis capable of being tested by measurements of the instability constants of a series of tervalent metal complexes with oxine and methyloxines variously substituted. It seems reasonable to suppose that with 2-substituents bulkier than methyl the energy barrier imposed by steric factors might be

sufficient to prevent the formation of, *e.g.*, aluminium, chromium, and gallium complexes, whilst permitting those of indium and thallium. For the present the development of such reagents which would owe their greater selectivity to controlled steric hindrance must proceed empirically.

Steric hindrance to complex formation of the type discussed in the case of 2-methyloxine must occur also when the interfering group forms part of an attached ring system, as shown by the failure of the hydroxyacridines (II) and (III) to give insoluble complexes with aluminium. Our speculations are strengthened by interesting results obtained by Freeman and Lions (*loc. cit.*) in a preliminary study of the potentialities of 1-hydroxyacridine (II) as an analytical reagent, though they did not interpret them. It was found that ferrous and ferric ions gave the same 2 : 1 complex (IV); presumably steric hindrance does not permit the formation of the expected 3 : 1 complex, but reduction of ferric ions by the phenolic reagent gives ferrous ions from which (IV) is obtained. The non-existence of a 3 : 1 complex from chromic ions is likewise to be expected; but the formation of an analogue of (IV) is equally unlikely in view of the difficulty of reducing Cr^{+++} to Cr^{++}. It is thus of great interest that the complex actually obtained



(IV.) (V.) (VI.) (VII.)

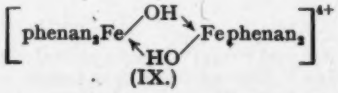
could be formulated as (V). In (IV) and (V) the cyclic ligands need not be coplanar and do not interfere sterically with one another. The soluble complex formed by aluminium and 2-methyloxine, and aluminium in the presence of a deficiency of oxine (page 1492) may well be similarly constituted. That certain metals do react with 2-methyloxine, thereby displacing protons, is demonstrable by electrometric titration, and we will discuss the exact constitution of these complexes in the sequel. We have confirmed Freeman and Lions's statement that aluminium and bismuth are not precipitated by 1-hydroxyacridine, but disagree in obtaining an orange-red precipitate with stannous tin in (hot) test solutions *A* and *B*. In addition to those cases mentioned in Table I, 9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine (III) gave yellow complexes with Bi^{+++}, Pb^{++}, Hg^{++}, Co^{++}, and Ni^{++}, and a black precipitate with Ag^{+}. The anhydrous *zinc* complex, $Zn(C_{13}H_{12}ON)_2$, was readily obtained pure. Gallium is incompletely precipitated by 1-hydroxyacridine, and the composition of the basic *precipitate* agrees most closely with that of the 1 : 1 complex, $[Ga(C_{13}H_8ON)(OH)_2(H_2O)_2]$.

The relation between 8-hydroxyquinoline (I; R = H) and 1-hydroxyacridine (II) is paralleled by that between picolinic acid (VI) and quinaldinic acid (VII). The steric factors which inhibit 3 : 1 complex formation with the hydroxyacridine (II) may likewise be expected to operate with quinaldinic acid, and it is significant that, whilst the latter forms normal 2 : 1 complexes of considerable analytical importance with cadmium, copper, zinc, and other bivalent metals, the complexes with aluminium and chromium are described as "basic" (Flagg, "Organic Reagents," New York, 1948, 246) and their compositions are probably based on, *e.g.*, (V) and (VIII) (see below). Picolinic acid, on the other hand, forms the expected 3 : 1 complexes with tervalent iron, chromium, and cobalt of type, $M(C_6H_4O_2N)_3$, as well as basic complexes such as $Fe(C_6H_4O_2N)_2OH$ (VIII; Ley and Ficken, *Ber.*, 1917, **50**, 1133; Ley, Schwarte, and Münnich, *Ber.*, 1924, **57**, 355; Gorvin, *J.*, 1944, 25). A compound with aluminium does not seem to have been described, and no study has as yet been published of the complexes of 6-substituted picolinic acids or 3-substituted *iso*quinoline-1-carboxylic acids which, as analogues of 2-substituted oxines, might be expected to reveal the influence of steric factors on the ease of complex formation.

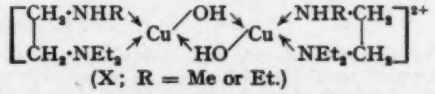
Failure to obtain a *precipitate* of the expected composition has hitherto provided the only indication of the operation of steric factors in the particular examples of the uncharged inner complexes discussed above, but steric factors may be equally important in the formation and stability of hydrophilic complexes carrying a net charge—where the simple diagnostic of solubility fails. That this is in fact the case is seen in the reactions of polypyridyls and *o*-phenanthrolines with ferrous ions, which have been extensively studied on account of their analytical importance both in the colorimetric estimation of iron and as oxidation–reduction indicators. Though 2 : 2′-dipyridyl and *o*-phenanthroline give red tris-bidentate complexes, steric hindrance operates to reduce the colour intensity with 3 : 3′-dimethyl-2 : 2′-dipyridyl

(Cagle and Smith, *J. Amer. Chem. Soc.*, 1947, **69**, 1860), 6-substituted 2 : 2′-dipyridyls (Burstall, *J.*, 1938, 1664), and 2-methyl-1 : 10-phenanthroline (Pfeiffer and Christeleit, *J. pr. Chem.*, 1938, **151**, 127), and completely inhibits complex formation in the cases of many 6 : 6′-disubstituted 2 : 2′-dipyridyls (Burstall, *loc. cit.*), 2-2′-quinolylpyridine, and 2 : 2′-diquinolyls (Smirnoff, *Helv. Chim. Acta*, 1921, **4**, 802). Similar behaviour in the case of bivalent kations which give colourless complexes with substituted dipyridyls and analogous ligands can be inferred from their ultraviolet absorption spectra or from their influence upon the spectra of other, coloured complexes, or more directly by measurements of their consecutive instability constants. The extent to which steric factors modify the stability of such complexes and cause departure from the " natural sequence " of stability already noted among different metals with varied ligands (Mellor and Maley, *Nature*, 1947, **159**, 370; 1948, **161**, 436; Irving and Williams, *ibid.*, 1948, **162**, 764) will be discussed in later papers.

Where 3 : 1 complexes are sterically inhibited, an alternative solution to that adopted in the case of, *e.g.*, chromium and 1-hydroxyacridine [page 1494 and formula (V)] is illustrated by the case of ferric ions and *o*-phenanthroline (phenan = $C_{12}H_8N_2$). Red tris-*o*-phenanthroline–ferrous ions, $[Fe\ phenan_3]^{++}$ are oxidised with difficulty to feebly-blue tris-*o*-phenthroline–ferric ions, $[Fe\ phenan_3]^{+++}$; the change is reversible with an oxidation potential of 1·14 volts. When, however, phenanthroline is allowed to react directly with ferric ions, the above tris-complex is not formed, but the binuclear complex (IX) results. An analogous case is provided by cupric ions, which react normally with ethylenediamine, *N*-methyl-, and *NN′*-diethyl ethylenediamine to give 2 : 1-complexes, whilst *N*-methyl (or -ethyl)-*N′*-diethylethylenediamine gives the binuclear complex (X; R = Me or Et).



(IX.)



(X; R = Me or Et.)

It is worth noting that simple 3 : 1 complexes of dimethylglyoxime (dmg = $C_4H_7O_2N_2$), or of sulphamide, $NH_2{\cdot}SO_2{\cdot}NH_2$, appear to be incapable of existence (cf. Mann, *J.*, 1933, 412). This explains the remarkable result of great analytical importance that, for the two so closely related elements cobalt and nickel, *o*-nitrosophenols are specific reagents for the former and dialkylglyoximes and analogously constituted substances for the latter. For, though the stability of complexes increases along the transition series Fe^{++}, Co^{++}, Ni^{++}, and Cu^{++}, with cobaltous complexes less stable than those of bivalent nickel (Irving and Williams, *loc. cit.*) the former is readily oxidised to the tervalent state by the phenolic reagent and in this state forms a far more stable complex (cf. the sequence $[Co\ en_3]^{+++} \gg [Ni\ en_3]^{++} > [Co\ en_3]^{++}$ for which the decadic logarithms of the instability constants are −48·7, −18·6, and −13·8, respectively; Bjerrum, " Metal Ammine Formation in Aqueous Solution," Copenhagen, 1941). On the other hand, whilst nickel forms with dimethylglyoxime an insoluble, planar inner-complex, $[Ni(dmg)_2]$, which carries no net charge, the corresponding cobaltous complex would have an unpaired 5*d* electron. After oxidation to the cobaltic state, the formation of a tris-complex with dimethylglyoxime is presumably hindered sterically, and instead there must result soluble complexes of the type, $[Co(dmg)_2(NH_3)_2]^+Cl^-$, studied by Tschugaieff (*Z. anorg. Chem.*, 1905, **46**, 144; *Ber.*, 1908, **41**, 2226).

Craig (*J.*, 1946, 534) has shown that the feebly basic character of the ring nitrogen atom in 1-amino-9-methyl- (or 1 : 9-diamino-)acridine can be explained in terms of steric hindrance by the 1 and 9 substituents to the approach of oxonium ions. The presence of a 7-substituent in a tris-8-hydroxyquinoline complex (cf. Fig.) may similarly (if less effectively) restrict the accession of oxonium ions and so permit the complex to persist in solutions of greater acidity. It is suggested that this explains, at least in part, the enhanced stability of 8-hydroxy-7-methylquinoline (page 1492) and the stability towards acid of the metal complexes of 5 : 7-dibromo-(or -dichloro)-8-hydroxyquinoline, 7-iodo-8-hydroxyquinoline-5-sulphonic acid, and the 7-arylazo-8-hydroxy-5-methylquinolines mentioned above (page 1489).

A final aspect of steric hindrance remains to be mentioned. Albert *et al.* (*Brit. J. Exp. Path.*, 1947, **28**, 69) have advanced the hypothesis that the bacteriostatic action of certain drugs arises from their ability to chelate with certain trace-metals present in parts of enzyme systems, thus interfering with the metabolic cycle of the bacterium. They showed that 2-methyloxine, 1-hydroxyacridine, and 1-hydroxyphenazine were less effective drugs than oxine [which did not differ greatly from 5-methyl- or 5-propyl-oxine or 8-hydroxypyridino(2′ : 3′-5 : 6)quinoline] and suggested that substitution adjacent to the nitrogen atom of the oxine ring

system caused steric hindrance to such chelation. Similar measurements have been carried out with some of the reagents tested above. The results, given in Table III, confirm the reduced activity of 1-hydroxyacridine (as compared with oxine) and establish similar behaviour for 2-phenyloxine. However, our figure for 2-methyloxine scarcely differs from that for oxine,

TABLE III.

Bacteriostatic Indexes.

Compound.	Albert *et al.*	This paper.
Oxine	100,000	102,400
2-Methyloxine	13,000	51,200
5-Methyloxine	51,000	—
5-Propyloxine	51,000	—
6-Methyloxine	—	51,200
7-Methyloxine	—	51,200
2-Phenyloxine	—	12,800
1-Hydroxyacridine	4,000	12,800
9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine	—	102,400

The bacteriostatic index given represents the smallest concentration expressed as 1/molarity which completely inhibited the growth of *Staph. aureus* under the conditions of test used by Albert *et al.* (*loc. cit.*).

whilst that for the reduced hydroxyacridine is identical and shows no indication of the operation of steric factors in its bacteriostatic action. The problem is clearly too complex to warrant a further discussion of the meagre evidence at present available.

EXPERIMENTAL.

4-o-*Hydroxyanilopentan-2-one*.—Acetylacetone (5 g.; 0·05 mol.) and *o*-aminophenol (2·75 g.; 0·025 mol.) were heated for 4 hours on a water-bath under reflux. The resultant light-brown solid was rubbed with portions of ether (30 ml. in all) to remove unchanged starting material, and recrystallised from aqueous alcohol; 4-o-*hydroxyanilopentan-2-one* separated as light-buff-coloured needles, m. p. 184° (Found: C, 69·0; H, 7·1; N, 7·25. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·8; N, 7·3%). Attempts at ring closure by warming with 90% sulphuric acid at 100°, heating under reflux with phosphoric oxide in dry toluene, or heating with a phosphoric oxide–phosphoric acid mixture at 140° or under reflux with *o*-aminophenol hydrochloride and zinc chloride in ethyl alcohol gave unchanged starting materials.

Action of Phenyl-lithium on 8-Hydroxyquinoline.—Lithium (1·5 g.; 2 equivalents) was cut up and stirred under dry nitrogen with ether (200 ml.) during the addition of bromobenzene (31·5 g.). After 40 minutes the flask was cooled to 0° and 8-hydroxyquinoline (14·5 g.; one equivalent) in dry ether (300 ml.) was slowly added, whereupon the orange colour changed to lime-yellow. After 10 minutes, water (150 ml.) was added, and the solution neutralised partly with hydrochloric acid and finally with acetic acid. On steam-distillation ether and unchanged oxine were removed. The residue was acidified, boiled with animal charcoal, filtered, and neutralised. The slate-grey product gave on distillation at atmospheric pressure a pale yellow oil, b. p. 320—325°, which solidified on cooling. Recrystallisation from methyl alcohol gave 8-hydroxy-2-phenylquinoline (I; R = C_6H_5) as faintly yellow plates, m. p. 58—59° (Found: C, 81·3; H, 5·0. Calc. for $C_{15}H_{11}ON$: C, 81·45; H, 5·0%). The picrate (yellow needles from alcohol) had m. p. 158—159°. Doebner and Fettbach (*Annalen*, 1894, **281**, 9) give 59° and 152°, respectively. A sample prepared from 8-hydroxy-2-phenylquinoline-4-carboxylic acid by decarboxylation, following Doebner and Fettbach (*loc. cit.*), formed almost colourless needles, m. p. 85·5° after repeated crystallisation from ethyl alcohol (Found: C, 81·8; H, 5·0. Calc.: C, 81·45; H, 5·0%). Admixed with the sample, m. p. 58—59°, the mixed m. p. was 58° and the picrate, m. p. 159—160°, did not depress the melting point of the former specimen.

Ring-closure of 2-Methoxydiphenylamine-2′-carboxylic Acid.—(*a*) The diphenylamine (16 g.; Ullmann, *loc. cit.*) was refluxed at 120° with freshly distilled phosphorus oxychloride (90 ml.) until evolution of hydrogen chloride had ceased (2 hours). After removal of excess of the oxychloride by distillation under reduced pressure the viscous residue was triturated with ice–water and dilute ammonia. Crude 5-chloro-1-methoxyacridine was collected and at once hydrolysed by boiling for ½ hour with 5% hydrochloric acid (700 ml.). 1-*Methoxyacridone* which separated on cooling was collected (yield, 12·8 g.) and recrystallised from 50% aqueous acetic acid from which it separated in light-buff needles, m. p. 284—285° (Found: C, 74·5; H, 5·1; N, 6·3. $C_{14}H_{11}O_2N$ requires C, 74·6; H, 4·9; N, 6·3%). The acridone was insoluble in water and dilute acids and alkali. Solutions in glacial acetic acid and concentrated sulphuric acid showed a blue-green fluorescence.

(*b*) When ring-closure was attempted by heating 2-methoxydiphenylamine-2′-carboxylic acid with 75% sulphuric acid or various sulphuric–acetic acid mixtures at 105° for 15 minutes, the starting material was recovered unchanged. With concentrated sulphuric acid no reaction took place at room temperature (1 hour) or at 55° (20 minutes), but at 70° and 98° heating for 5—55 minutes gave a yellow *product*, m. p. 293° from 50% aqueous acetic acid; the properties agreed with those of Ullmann's "9-methoxyacridone." However, it contained sulphur, a typical analysis being C, 56·2; H, 3·5; N, 5·2 S, 10·7 [Calc. for $C_{14}H_{11}O_2N$: C, 74·6; H, 4·9; N, 6·3%. $C_{14}H_{10}O_2N(SO_3H)$ requires C, 55·1; H, 3·6; N, 4·6; S, 10·5%].

Reduction of 1-Methoxyacridone to 1-Methoxyacridine.—A solution of 1-methoxyacridone (11·3 g.

in 600 ml. of amyl alcohol) was boiled under reflux, and sodium (30 g.) was added gradually in small pieces until the initial fluorescence had vanished. The amyl alcohol was removed by steam-distillation and the resulting crude 1-methoxyacridane extracted with ether. After removal of the solvent, the sticky yellow residue was dissolved in alcohol (150 ml.) and oxidised by the slow addition of 2N-silver nitrate (30 ml.); the reaction was completed by gentle warming and, after the mixture had been kept overnight, excess of dilute hydrochloric acid was added, and the mixture filtered (after warming to coagulate the silver chloride). On basification, 1-methoxyacridine separated from the filtrate and gave light yellow needles (9 g.), m. p. 130°, from aqueous alcohol. Jensen and Rethwisch (*loc. cit.*) give m. p. 130—131°. Demethylation by boiling under reflux with hydrobromic acid (*d* 1·48) for 3—4 hours gave 1-hydroxyacridine (II) in 97% yield; m. p. 116—117° (from 75% ethyl alcohol).

Preparation of 9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine (III).—9-Methoxy-1 : 2 : 3 : 4-tetrahydroacridine (5 g.; Petrow, *J.*, 1942, 693) was heated under reflux for 3½ hours with hydrobromic acid (45 ml.; *d* 1·48). After removal of excess of acid by distillation under reduced pressure the residue was neutralised with sodium carbonate, and *9-hydroxy-1 : 2 : 3 : 4-tetrahydroacridine* (III) was collected (yield, 4·5 g.). It crystallised from alcohol–water in colourless needles, m. p. 89—90° (Found : C, 78·3; H, 6·5; N, 7·0. $C_{13}H_{13}ON$ requires C, 78·3; H, 6·6; N, 7·0%).

Testing of Reagents.—(a) *Composition of test solutions.* Solution *A* was prepared from 250 ml. of 2N-acetic acid, 225 g. of sodium acetate (trihydrate), and 500 ml. of distilled water. Solution *B* contained 40 ml. of 2N-ammonium hydroxide, 320 g. of ammonium acetate, 30 g. of sodium potassium tartrate, and 850 ml. of distilled water. Solution C contained 500 ml. of 2N-sodium hydroxide, 275 g. of sodium potassium tartrate, and 500 ml. of distilled water. Stock solutions of metals containing 0·01 g.-atom/l. were prepared from "Spekpure" metals or "AR" salts. Each organic reagent was used in the form of a 0·1M. solution in ethyl alcohol.

(b) *Procedure.* 4·0, 0·4, and 0·04 Ml. of 0·01N. metal solution were placed severally in three test-tubes (selected 6 × ½-inch tubes which had been previously boiled in nitric acid, repeatedly rinsed in distilled water, and finally cleaned and dried with live steam); 2 ml. of buffer *A*, 0·2 ml. of organic reagent, and sufficient water to make the total volume up to 6·2 ml. were then added. A fourth tube containing 2 ml. of buffer, 0·2 ml. of reagent, and 4 ml. of water, but no metal, served to determine the behaviour of the reagent, whilst an additional control tube containing 0·4 ml. of metal solution, 2 ml. of buffer, and 3·8 ml. of water, but no reagent, ensured that the possible precipitation of metal as hydroxide or basic salt under the conditions of testing employed could not be mistaken for complex formation. Each tube was then heated for 15 minutes at 80° and examined, when cold, for precipitation. If results were positive with as little as 0·04 ml. of 0·01N. metal solution, the whole series was repeated with 0·001N. metal solutions and, if necessary, with still more dilute solutions, until precipitation did not occur. The limits of sensitivity were then more exactly delimited by carrying through a series of tests with 0·4, 0·2, 0·1, and 0·04 ml. of metal solution of the appropriate strength. The sequence of testing was then repeated with buffer solutions *B* and *C*, in turn, and the whole procedure repeated for each reagent and every metal studied. In a few cases the reagent was insoluble in the cold buffer, though freely soluble at 80°, whilst the metal complex was sufficiently insoluble for its formation to be detected with certainty when observations were made on the hot solution. In some cases, where the reagent was insoluble in a particular buffer both hot and cold, the colour or appearance of the metal complex was often sufficiently distinctive to enable its formation to be detected despite the presence of excess of reagent. Sensitivities obtained under these exceptional conditions are liable to have been under-estimated.

The Complex of Gallium and 8-Hydroxy-2-methylquinoline.—A solution of gallium chloride (0·0516 g. of metal) in 25 ml. 0·2N-hydrochloric acid was diluted to 250 ml., and a 5% excess of 2-methyloxine added in the form of a 2% alcoholic solution. A yellow colour developed. After heating to 60—70°, 2N-ammonia solution was added dropwise until the separation of a flocculent precipitate was complete. After digestion for 1½ hours, this became granular and was collected on a sintered-glass crucible, washed thoroughly to remove excess of reagent, and dried at 130° for 1 hour. The following weights of precipitate were obtained : 0·3759, 0·3763, 0·3828, 0·3885, and 0·3906 g.; calc. for $Ga(C_{10}H_8ON)_3$, 0·4025 g. The defect in weight might be caused by incomplete precipitation, losses during washing due to the solubility of the *complex*, or to the formation of a basic complex or of a stoicheiometric 3 : 1 complex mixed with gallium hydroxide. The last two alternatives were excluded by determining the amount of 2-methyloxine present in the heaviest precipitate. This was dissolved in 500 ml. of 2N-hydrochloric acid, and 50 ml. containing 1 g. of potassium bromide aliquots were titrated with M/60-bromate, any excess over that required to form 2-methyl-5 : 7-dibromo-8-hydroxyquinoline being determined iodometrically [Found : $C_{10}H_8ON$, 87·2. $Ga(C_{10}H_8ON)_3$ requires 87·2%]. The procedure was first checked against pure 2-methyloxine. It is noteworthy that hot water was used to wash the first two precipitates and cold water for the others. Like the gallium–oxine complex (Geilmann and Wrigge, *Z. anorg. Chem.*, 1932, **209**, 129), the 2-methyloxine complex appears to be appreciably soluble in cold water.

The Complex of Indium and 8-Hydroxy-2-methylquinoline (with Miss M. Tutt.)—A yellow precipitate formed when 2N-sodium acetate solution was added to 150 ml. of a solution of indium (30—40 mg.) in dilute acid containing a 2% excess of 2-methyloxine. Addition of ammonia produced a further precipitate and after digestion at 70° for 1 hour it was collected, washed with the acid wash-liquor recommended by Berg (*Z. anorg. Chem.*, 1932, **204**, 208), and dried at 135°. The low results (Found : 0·1385, 0·1833, and 0·1688 g. Calc. : 0·1953 g.) were partly due to the solubility of the precipitate in the acid wash-liquor, and partly to the co-precipitation of indium hydroxide since the pH of the filtrate was found to be 5·5 or greater. Subsequent samples, made by adding ammonia dropwise to an indium solution containing 1 g. of sodium potassium tartrate, weighed 0·1951 and 0·1938 (calc., 0·1953 g.), 0·1935 and 0·1936 (calc., 0·1956 g.), and 0·2034 and 0·2023 g. (calc., 0·2031 g.). Though quantitative precipitation and recovery of the *complex* was not attained, its composition was established as before by bromination [Found : $C_{10}H_8ON$, 80·6. $In(C_{10}H_8ON)_3$ requires $C_{10}H_8ON$, 81·5%].

The Complex of Zinc and 9-Hydroxy-1 : 2 : 3 : 4-tetrahydroacridine.—A 3% excess of an alcoholic solution of the reagent was added to 50 ml. of zinc sulphate solution (containing 0·0525 g. of metal)

acidified with 5 ml. of 2N-hydrochloric acid. After addition of 2 g. of ammonium acetate and heating to 80—90°, 2N-ammonium hydroxide was added dropwise until precipitation was complete. After digestion at 90° for 1 hour, the precipitate was collected, washed with hot water, and dried at 135° [Found: 0·3593, 0·3524, and 0·3554 g.; C, 67·4; H, 5·1; N, 6·2; Zn, 14·2. $Zn(C_{13}H_{12}ON)_2$ requires C, 67·6; H, 5·2; N, 6·1; Zn, 14·2%].

The Complex of Gallium and 1-Hydroxyacridine.—0·0205 G. of gallium was precipitated with 1-hydroxyacridine as described above for the zinc complex. The weights of precipitate were 0·1060, 0·0981, and 0·0895 g., and a typical analysis gave C, 53·9; H, 3·6; Ga, 22·6%.

The Complex of (Tervalent) Thallium and 5:7-Dibromo-8-hydroxyquinoline (with E. J. Risdon).—Thallium (15 mg.), as nitrate, in 100 ml. of 0·01N-hydrochloric acid was oxidised by 15—30 ml. of bromine water. After heating to 50°, dibromo-oxine (80 mg. in 150 ml. of acetone) was added and 2N-ammonium hydroxide run in dropwise until precipitation was complete (pH ~8·6). The solution was digested for 1 hour and cooled, and the yellow complex collected and dried (at 120°). In successive experiments it weighed 78·4 and 78·6 mg. [Calc. for $Tl(C_9H_5ONBr_2)_3$: 81·5 mg.].

Bacteriological Tests (with Miss M. N. Jennings).—Oxine and other reagents were tested, by the serial-dilution method, for their power to inhibit the growth of one strain each of *Staph. aureus* and *Bact. coli*. Aqueous M/80 solutions of each substance, after being sterilised in the autoclave, were diluted in two-fold series, and 0·2 ml. of each dilution was added to 1·8 ml. of nutrient broth. The tubes were inoculated with 1 drop of a 1 in 1,000 dilution of an overnight culture of the organism. The results were observed after incubation overnight. As already noted by Albert *et al.* (*loc. cit.*), oxine and its analogues proved to be relatively ineffective towards *Bact. coli*.

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318. 4-*Aminosalicylic Acid and Its Derivatives.*

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The acid obtained by carboxylation of 3-aminophenol is shown to be 4-aminosalicylic acid. A synthesis of the isomeric 4-hydroxyanthranilic acid is described. A number of derivatives of 4-aminosalicylic acid has been prepared for test against pathogenic strains of *M. tuberculosis*.

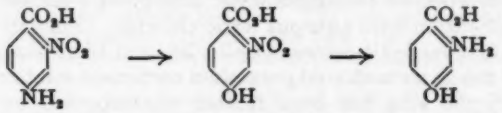
Following upon Bernheim's observation (*Science*, 1940, **92**, 204; *J. Bact.*, 1941, **41**, 387) that derivatives of benzoic and salicylic acids cause considerable increase in the oxygen uptake of tubercle bacilli, Lehmann (*Lancet*, 1946, I, 14, 15) examined a number of derivatives of benzoic acid in order to test their inhibitory effect upon the growth of tubercle bacilli. Of the compounds tested, 4-aminosalicylic acid was the most interesting, *in vitro* tests showing 50—75% inhibition of growth at a dilution of 1 : 650,000. This early promise was supported by limited clinical trial. Subsequent experience has shown that Lehmann's original claim is well founded, and that a *prima facie* case is established for a detailed clinical examination of the acid and its derivatives. This paper is a preliminary report upon the chemistry of 4-aminosalicylic acid, which was undertaken with the object of preparing derivatives of the acid for test against pathogenic strains of the tubercle bacillus; a study of the inhibitory effects of some of the derivatives described in this paper, has been reported (Goodacre, Mitchell, and Seymour, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 301). In addition, a convenient method for the preparation of the acid is described; a preliminary note upon the development of this method appeared in *Nature* (1948, **161**, 435) and this has been followed by a number of publications from other laboratories describing the same method (Sheehan, *J. Amer. Chem. Soc.*, 1948, **70**, 1665; Erlenmeyer, Prijs, Sorkin, and Suter, *Helv. Chim. Acta*, 1948, **31**, 988; Rosdahl, *Sartryck ur Svensk. Kemisk Tidsk.*, 1948, **60**, 12; Wessely, Benedikt, and Benger, *Monatsh.*, 1948, **79**, 185).

4-Aminosalicylic acid was first described by Seidel and Bittner (*Monatsh.*, 1902, **23**, 423), who prepared it by the reduction of 4-nitrosalicylic acid by means of tin and hydrochloric acid. We find that the reduction can be effected by using ferrous sulphate, Raney alloy and alkali, or hydrogen with a platinum catalyst.

The doses of 4-aminosalicylic acid used for clinical cases are relatively large (15—30 g. per day). The preparation of 4-aminosalicylic acid *via* 4-nitrosalicylic acid does not lend itself to large-scale application. Alternative methods for the preparation of 4-aminosalicylic acid were therefore sought. Of the methods examined, direct carboxylation of 3-aminophenol proved most successful. Treatment of 3-aminophenol with sodium or potassium hydrogen carbonate,

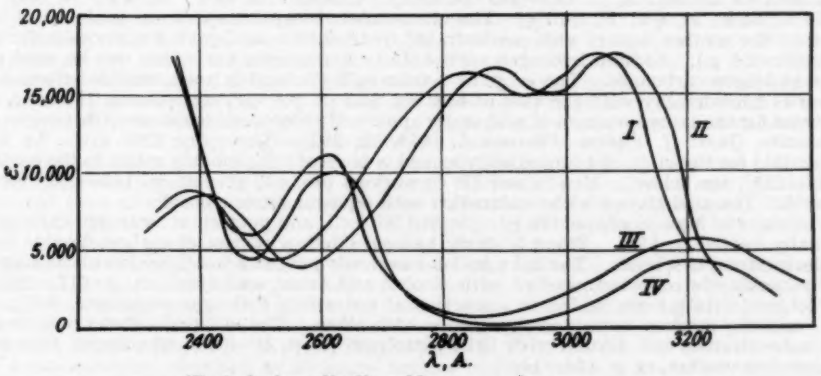
or ammonium carbonate, and carbon dioxide at 100—140° gave an aminohydroxybenzoic acid in yields of 40—50%.

This acid is most probably either 4-aminosalicylic acid or 4-hydroxyanthranilic acid. The latter structure was excluded as follows: An unambiguous synthesis of 4-hydroxyanthranilic acid was completed (cf. Rodionow and Fedorowa, *Bull. Soc. chim.*, 1939, **6**, 478) starting from 2-nitro-4-aminobenzoic acid (Schofield and Simpson, J., 1945, 512; Bogert and Kropff, *J. Amer. Chem. Soc.*, 1909, **31**, 841). The latter was converted into *2-nitro-4-hydroxybenzoic acid*, catalytic reduction of which gave *4-hydroxyanthranilic acid* characterised as its *hydrochloride*:



Methyl and *ethyl* 4-*hydroxyanthranilate* were prepared by similar reduction of *methyl* and *ethyl* 2-*nitro*-4-*hydroxybenzoate*. 4-Hydroxyanthranilic acid is different from the acid obtained by carboxylation of 3-aminophenol. The latter acid was converted into its *methyl* and *ethyl* esters, and these proved to be different from the corresponding esters of 4-hydroxyanthranilic acid.

Final proof that the acid obtained by carboxylation of 3-aminophenol is 4-aminosalicylic acid was obtained by comparison of its methyl, ethyl, n-*propyl*, n-*butyl*, and iso*butyl* esters with the esters prepared by reduction of the corresponding *esters* of 4-nitrosalicylic acid. Corresponding members of the two series of esters were identical.



(*I*) 4-*Aminosalicylic acid.*
(*II*) *Methyl 4-aminosalicylate.*
(*III*) 4-*Hydroxyanthranilic acid.*
(*IV*) *Methyl 4-hydroxyanthranilate.*
} *In ethanol.*

4-Aminosalicylic acid exhibits a characteristic intense absorption spectrum in the ultra-violet, with maxima at approximately 2390, 2840, and 3040 A.; methyl 4-aminosalicylate shows a similar absorption spectrum. 4-Hydroxyanthranilic acid and its methyl ester, on the other hand, exhibit maxima at approximately 2600 and 3200 A. (see Fig.). Way, Smith, Howie, Weiss, and Swanson (*J. Pharmacol.*, 1948, **93**, 368) have described the ultra-violet absorption spectrum of 4-aminosalicylic acid "obtained from different commercial houses". For this material they observed maxima at 2660 and 2980 A. Although the nature of the solvent used for the determination is not stated, the values given by these authors are demonstrably not those of 4-aminosalicylic acid since they also report that an ether extract of the sample of acid employed exhibits an absorption spectrum which is quite different from that of the original sample. The absorption spectrum of the ether extract appears to be similar to that of pure 4-aminosalicylic acid shown in the figure, but a close comparison is not possible since the solvent employed for the measurement of the ultra-violet absorption spectrum of the ether extract is not disclosed, nor is the concentration employed. We find exact correspondence in the absorption spectra of specimens of 4-aminosalicylic acid prepared by carboxylation of 3-aminophenol and by reduction of 4-nitrosalicylic acid.

After the method for the preparation of 4-aminosalicylic acid described above had been developed, we found a reference in Beilstein (4th Edn., **14**, 592) to "2-amino-4-hydroxybenzoic acid (?), m. p. 148° with decomp. to carbon dioxide and *m*-aminophenol," obtained by treatment

of 3-aminophenol with ammonium carbonate solution. Reference to the patent cited by Beilstein (D.R.-P. 50,835) showed that this structure was not ascribed to the reaction product which was simply described as "*m*-aminophenol carboxylic acid."

Acetylation of 4-aminosalicylic acid using one molecular proportion of acetyl chloride gives 4-acetamidosalicylic acid, which gives a positive reaction with aqueous ferric chloride. After oral administration of sodium 4-aminosalicylate, this compound has been isolated from human urine (cf. Bray, Ryman, and Thorpe, *Nature*, 1948, **162**, 64) and from the urine of rabbits (Venkataraman, Venkataraman, and Lewis, *J. Biol. Chem.*, 1948, **173**, 641). Using an excess of acetic anhydride, acetylation of 4-aminosalicylic acid gives *4-acetamido-2-acetoxybenzoic acid* which does not give a coloration with aqueous ferric chloride. Similarly, acetylation of methyl 4-aminosalicylate gives first *methyl 4-acetamidosalicylate* and then *methyl 4-acetamido-2-acetoxybenzoate*. Treatment of the latter with cold potassium carbonate solution gives the 4-monoacetyl derivative. 4-Aminosalicylic acid has been further characterised by the preparation of its *amide*, *hydrazide*, and 3 : 5-*dibromo*-derivative. These and other derivatives are described in the Experimental section.

Experimental.

4-Aminosalicylic Acid.—(i) A mixture of 3-aminophenol (40 g.), potassium hydrogen carbonate (110 g.), and water (260 c.c.) in an autoclave was treated with carbon dioxide (initial pressure, 5 atm.) and then heated for 6 hours at 100°. After filtration, the cold mixture was adjusted to pH approx. 6·5 by addition of hydrochloric acid, and then continuously extracted with ether. Evaporation of the extract gave 3-aminophenol (20·2 g.). The aqueous solution (charcoal) was acidified with acetic acid. The solid (18·8 g.) was collected and crystallised from aqueous methanol, from which 4-aminosalicylic acid separated as needles, m. p. 142—143° (decomp.) (Found: C, 55·1; H, 4·6; N, 8·9. Calc. for $C_7H_7O_3N$: C, 54·9; H, 4·6; N, 9·15%) The ultra-violet absorption spectrum is shown in the figure. Treatment of the mother-liquors with concentrated hydrochloric acid gave 4-aminosalicylic acid as its hydrochloride (2·5 g.). Sodium hydrogen carbonate or ammonium carbonate can be used instead of potassium hydrogen carbonate. The m. p. of 4-aminosalicylic acid is not a reliable criterion of purity since it varies considerably with the rate of heating, and m. p.s varying between 140° and 150° have been observed for the same specimen of acid under apparently identical conditions (Oberweger, Seymour, and Simmonite, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 292). The value 220° given by Seidel and Bittner (*loc. cit.*) for the m. p. of 4-aminosalicylic acid is in error; it probably refers to the hydrochloride (m. p. 224—225°, see below). Kondo and his co-workers (*loc. cit.*) give m. p. 145—151° for 4-aminosalicylic acid. The acid gives a violet coloration with aqueous ferric chloride.

(ii) A mixture of 3-aminophenol (20 g.), glycerol (80 c.c.), and potassium hydrogen carbonate (80 g.) was gradually heated to 130°. When frothing had subsided, a stream of carbon dioxide was passed through the mixture for 4 hours. The hot solution was slowly poured into 5% hydrochloric acid (500 c.c.), and the hydrochloride collected, washed with alcohol and ether, and dried (m. p. 217—222°; 16 g.). The hydrochloride (16 g.) was added to a solution of potassium hydrogen carbonate (8·4 g.) in water (100 c.c.), and the mixture extracted repeatedly with ether. The ethereal extract was treated with charcoal, concentrated, and diluted with light petroleum (b. p. 40—60°), whereupon 4-aminosalicylic acid separated as needles, m. p. 138—140°.

(iii) A solution of 4-nitrosalicylic acid (m. p. 235—236° decomp.) (18·3 g.) in a mixture of ammonia (*d* 0·88; 140 c.c.) and water (140 c.c.) was added slowly to a boiling solution of hydrated ferrous sulphate (195 g.) in water (400 c.c.). The solution was kept alkaline by addition of aqueous ammonia during the course of the reaction. When the addition was complete, the mixture was boiled for 10 minutes. The hot solution was filtered, and the filtrate concentrated to approximately half bulk under reduced pressure in an atmosphere of nitrogen. The cold solution was acidified to Congo-red with concentrated hydrochloric acid, and the crystalline hydrochloride was collected and washed with alcohol and acetone. The hydrochloride was treated with excess of sodium hydrogen carbonate solution, and the solution filtered (charcoal) and acidified with acetic acid. 4-Aminosalicylic acid separated as needles, m. p. 142—143° (decomp.) (yield 60—70%) (Found: C, 54·8; H, 4·5; N, 9·2. Calc. for $C_7H_7O_3N$: C, 54·9; H, 4·6; N, 9·15%).

The *hydrochloride* can be obtained by treatment of a solution of the acid in dry acetone or ether with dry hydrogen chloride. It is obtained as scintillating needles, m. p. 224—225° (Found: C, 44·8; H, 4·2; N, 7·6; Cl, 18·4. $C_7H_8O_3NCl$ requires C, 44·3; H, 4·2; N, 7·4; Cl, 18·7%).

(iv) A solution of 4-nitrosalicylic acid (1·8 g.) in dry ethyl acetate (50 c.c.) was shaken with hydrogen in the presence of platinum (from 100 mg. of PtO_2, H_2O) for two hours, hydrogen absorption (740 c.c. at 18° and 750 mm.) then being complete. The mixture was filtered, and the filtrate evaporated to dryness in an atmosphere of carbon dioxide. 4-Aminosalicylic acid (1·5 g., m. p. 140°) was crystallised from aqueous methanol, from which it separated as needles, m. p. 137—138° (Found: N, 9·3. Calc. for $C_7H_7O_3N$: N, 9·15%). Light absorption in alcohol: Maxima at 2390 A., $\varepsilon = 6800$; 2850 A., $\varepsilon = 14{,}800$; 3040 A., $\varepsilon = 16{,}400$.

(v) A solution of 4-nitrosalicylic acid (5 g.) in 10% sodium hydroxide (150 c.c.) was treated at 90° with Raney alloy (15 g.) added during 20 minutes, and the mixture heated for an hour at the same temperature. The hot mixture was filtered, and the cold filtrate poured into concentrated hydrochloric acid (110 c.c.) with stirring and cooling. The hydrochloride was collected and washed with alcohol and then had m. p. 224—225° (decomp.) undepressed when mixed with the specimens described above (yield, 60%) (Found: C, 44·5; H, 4·6; N, 7·3%).

Decomposition of the hydrochloride as described above gave 4-aminosalicylic acid, m. p. 141·5° (decomp.) undepressed when mixed with the previously described specimens.

4-Aminosalicylamide.—A solution of 4-nitrosalicylamide (1 g.) in ethanol (100 c.c.) was shaken with hydrogen in the presence of platinum from platinum oxide (50 mg.) for 3 hours at room temperature, gas absorption then being complete. The product was recrystallised (charcoal) from hot water, from which *4-aminosalicylamide* (0·75 g.) separated as hexagonal plates, m. p. 160—161° (Found: C, 55·2; H, 5·3; N, 18·4. $C_7H_8O_2N_2$ requires C, 55·3; H, 5·3; N, 18·4%).

4-Aminosalicylhydrazide.—Methyl 4-aminosalicylate (4·0 g.) was heated on the water-bath with hydrazine hydrate (90%, 3 c.c.) for 30 minutes. More hydrazine hydrate (2 c.c.) was added, and heating continued for 1 hour. The mixture was diluted with water, and the solid (1·8 g.) crystallised from aqueous ethanol to yield *4-aminosalicylhydrazide* as felted needles, m. p. 197° (decomp.) (Found: C, 50·3; H, 5·5; N, 24·9. $C_7H_9O_2N_3$ requires C, 50·3; H, 5·4; N, 25·1%).

4-Acetamidosalicylic Acid.—(*a*) A solution of 4-aminosalicylic acid (1·53 g.) in pyridine (8 c.c.) was heated at 100° for 2 hours with acetyl chloride (0·84 g.). The cold solution was poured on a mixture of ice and hydrochloric acid, and the mixture filtered. The solid (1·5 g., m. p. 220—222°, decomp.) was crystallised from glacial acetic acid, from which 4-acetamidosalicylic acid separated as a microcrystalline powder, m. p. 220—222°. The m. p. varies with the rate of heating; with rapid heating, it is 232° (Found: C, 55·1; H, 5·2; N, 7·1. Calc. for $C_9H_9O_4N$: C, 55·3; H, 4·6; N, 7·2%). With aqueous ferric chloride the acid gives a purple coloration. Light absorption in alcohol: Maxima at 2690 A., $\varepsilon = 19{,}200$; 3040 A., $\varepsilon = 9100$.

(*b*) A 24-hour specimen of urine (*ca.* 750 c.c.), collected during oral administration of sodium 4-aminosalicylate (25 g. per day) to a woman, was adjusted to pH approx. 5·0 by addition of hydrochloric acid and continuously extracted with ether for 48 hours. The extract was washed with 10% hydrochloric acid, dried (Na_2SO_4), and evaporated. The solid residue was dissolved in cold sodium hydrogen carbonate solution, the mixture filtered, and the filtrate acidified with dilute hydrochloric acid. The solid (2 g.) was crystallised from glacial acetic acid, from which 4-acetamidosalicylic acid separated as microprisms, m. p. 224° undepressed when mixed with the specimen described above (Found: C, 55·5; H, 4·8; N, 7·1%). Light absorption in ethanol: Maxima at 2690 A., $\varepsilon = 17{,}000$; 3050 A., $\varepsilon = 7000$.

4-Acetamido-2-acetoxybenzoic Acid.—4-Aminosalicylic acid (3·06 g.) was heated at 100° with acetic anhydride (6 c.c.) for 1 hour. The mixture was diluted with water, and the solid collected and crystallised from glacial acetic acid, from which *4-acetamido-2-acetoxybenzoic acid* separated as needles, m. p. 189—190·5° (Found: C, 55·4; H, 4·6; N, 6·2. $C_{11}H_{11}O_5N$ requires C, 55·7; H, 4·6; N, 5·9%).

4-Benzamidosalicylic acid.—4-Aminosalicylic acid (6·1 g.) in aqueous sodium hydroxide (20%; 20 c.c.) was treated with benzoyl chloride (5·6 g.) added slowly with shaking. After acidification with mineral acid, the solid was collected and crystallised from aqueous ethanol, from which *4-benzamidosalicylic acid* separated as needles, m. p. 253°. It gives a purple colour with ferric chloride (Found: C, 65·5; H, 4·2; N, 5·4. $C_{14}H_{11}O_4N$ requires C, 65·4; H, 4·3; N, 5·45%).

4-Succinamidosalicylic Acid.—A solution of 4-aminosalicylic acid (3 g.) in acetone (60 c.c.) was refluxed with succinic anhydride (2·6 g.) for 1½ hours. The solid was collected and crystallised from aqueous ethanol, from which 4-succinamidosalicylic acid separated as a *monohydrate*, m. p. 230° (decomp.) (Found: C, 48·7; H, 4·5; N, 5·2; equiv., 275. $C_{11}H_{11}O_6N,H_2O$ requires C, 48·7; H, 4·8; N, 5·2%; equiv., 271). It gives a purple coloration with aqueous ferric chloride.

3 : 5-Dibromo-4-aminosalicylic Acid.—A solution of 4-aminosalicylic acid (4·6 g.) in glacial acetic acid (90 c.c.) was treated at room temperature with a solution of bromine (9·6 g.) in glacial acetic acid (120 c.c.). The solid separating (5·3 g.) was triturated with water and crystallised from 50% acetic acid, from which *3 : 5-dibromo-4-aminosalicylic acid* separated as needles, m. p. 205° (decomp.) (Found: C, 27·0; H, 1·6; N, 4·1; Br, 51·5. $C_7H_5O_3NBr_2$ requires C, 27·0; H, 1·6; N, 4·5; Br, 51·4%).

Methyl 4-nitrosalicylate, obtained by 20 hours' heating under a reflux of a solution of 4-nitrosalicylic acid (5·0 g.) with methanol (100 c.c.) either containing concentrated sulphuric acid (5 c.c.) or saturated with dry hydrogen chloride, separates from aqueous methanol as elongated yellow plates and from light petroleum (b. p. 60—80°) as yellow prisms, m. p. 99·5—100° (Found: C, 49·0; H, 3·7; N, 7·5. $C_8H_7O_5N$ requires C, 48·7; H, 3·55; N, 7·1%). Ethyl 4-nitrosalicylate was obtained by the method described by Borsche (*Annalen*, 1912, **390**, 18); it separates from ethanol as needles, m. p. 87°.

The following 4-nitrosalicylic esters were obtained by the same methods. n-*Propyl*, flat yellow needles from aqueous *n*-propanol, m. p. 32—33° (Found: C, 53·5; H, 5·1; N, 6·3. $C_{10}H_{11}O_5N$ requires C, 53·3; H, 4·9; N, 6·2%); iso*propyl*, pale yellow needles, m. p. 72°, from aqueous *iso*propanol (Found: C, 53·3; H, 4·7; N, 6·5%); n-*butyl*, pale yellow needles, m. p. 34°, from methanol (Found: C, 55·4, 55·4; H, 5·7, 5·5; N, 6·0. $C_{11}H_{13}O_5N$ requires C, 55·2; H, 5·4; N, 5·9%); iso*butyl*, pale yellow needles, m. p. 62—63°, from light petroleum (Found: C, 55·1; H, 5·3; N, 5·8%).

2-Hydroxyethyl 4-nitrosalicylate (obtained by the sulphuric acid method) separates from benzene as pale yellow needles, m. p. 121—122° (Found: C, 47·5; H, 4·0; N, 6·5. $C_9H_9O_6N$ requires C, 47·5; H, 4·0; N, 6·2%).

Methyl 4-Aminosalicylate.—(*a*) A solution of 4-aminosalicylic acid [prepared by method (i) above; 1·5 g.] in dry methanol (40 c.c.) and concentrated sulphuric acid (2 c.c.) was heated under reflux for 8 hours. The solution was concentrated to a bulk of 10 c.c. and neutralised by addition of 10% sodium carbonate solution. The solid was collected and crystallised from aqueous methanol, from which *methyl 4-aminosalicylate* (0·75 g.) separated as needles, m. p. 120—121° (Found: C, 57·3, 57·3; H, 5·5, 5·4; N, 8·6. $C_8H_9O_3N$ requires C, 57·5; H, 5·4; N, 8·4%). The ultra-violet absorption spectrum is shown in the figure.

(*b*) A solution of 4-aminosalicylic acid in dry ether was treated with a solution of diazomethane (1 mole) in ether at 0° until nitrogen evolution ceased. The solution was washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Recrystallisation of the crystalline residue from light petroleum (b. p. 100—120°) gave the methyl ester as needles or plates, m. p. 119—120° either alone or mixed with the specimen described above (Found: C, 57·7; H, 5·8; N, 8·4%).

(*c*) A solution of methyl 4-nitrosalicylate (7·5 g.) in ethanol (250 c.c.) was shaken with hydrogen and platinum oxide until hydrogen absorption ceased. The filtered solution was evaporated, and the residue crystallised from light petroleum (b. p. 60—80°) to give methyl 4-aminosalicylate (4·5 g.) as needles, m. p. 119—120° undepressed when mixed with the specimens described above (Found: C, 57·3; H, 5·5;

N, 8·4, 8·6%). Light absorption in alcohol: Maxima at 2410 A., ε = 9000; 2890 A., ε = 15,600; 3070 A., ε = 18,000.

The following 4-aminosalicylates were prepared by suitable adaptation of methods (*a*) and (*c*) above. The esters obtained by the two methods are identical: *Ethyl*, needles, m. p. 111·5—113°, from light petroleum (b. p. 100—120°) or from aqueous acetone (Found: C, 59·5; H, 5·8; N, 7·5. $C_9H_{11}O_3N$ requires C, 59·7; H, 6·1; N, 7·7%); n-*propyl*, needles, m. p. 102—103°, from light petroleum (b. p. 60—80°) or from aqueous alcohol (Found: C, 61·7; H, 6·7; N, 6·8. $C_{10}H_{13}O_3N$ requires C, 61·5; H, 6·7; N, 7·2%); iso*propyl* [method (*c*) only], needles, m. p. 75—76°, from light petroleum (b. p. 60—80°) (Found: C, 61·8, 61·7; H, 6·8, 6·6; N, 7·3, 7·1%); n-*butyl*, needles, m. p. 92·5—93°, from light petroleum (b. p. 60—80°) or aqueous ethanol (Found: C, 63·2; H, 7·1; N, 6·6. $C_{11}H_{15}O_3N$ requires C, 63·2; H, 7·2; N, 6·7%); iso*butyl*, needles, m. p. 84°, from light petroleum (b. p. 60—80°) (Found: C, 63·3; H, 7·4; N, 6·8%).

n-*Amyl* 4-*Aminosalicylate*.—*n*-Amyl 4-nitrosalicylate was prepared by the method described for its homologues; it was obtained as an oil which was catalytically reduced without further purification. n-*Amyl* 4-*aminosalicylate* was obtained as plates, m. p. 70°, from light petroleum (b. p. 100—120°) (Found: C, 64·4; H, 7·5; N, 6·4. $C_{12}H_{17}O_3N$ requires C, 64·5; H, 7·6; N, 6·3%). 2-*Hydroxyethyl* 4-*aminosalicylate*.—This [method (*c*) only] formed plates, m. p. 130—131°, from water (Found: C, 54·7; H, 5·5; N, 7·2. $C_9H_{11}O_4N$ requires C, 54·9; H, 5·6; N, 7·1%).

Methyl 4-*Acetamidosalicylate*.—A solution of methyl 4-aminosalicylate (1·67 g.) in chloroform (20 c.c.) and acetic anhydride (1 c.c.) was heated under reflux for 2 hours. The mixture was evaporated under reduced pressure, and the residue crystallised from aqueous ethanol to yield *methyl* 4-*acetamidosalicylate* as needles, m. p. 150°, which gave a purple coloration with aqueous ferric chloride (Found: C, 57·7; H, 5·2; N, 6·9. $C_{10}H_{11}O_4N$ requires C, 57·4; H, 5·3; N, 6·7%). The ester was also obtained by treatment of 4-acetamidosalicylic acid in ethereal solution with diazomethane.

Methyl 4-*Acetamido*-2-*acetoxybenzoate*.—A solution of methyl 4-aminosalicylate (3·34 g.) in acetic anhydride (3·75 c.c.) and acetic acid (2 c.c.) was heated on the water-bath for 1 hour. The solution was poured into water, and the solid collected and crystallised from aqueous ethanol, from which *methyl* 4-*acetamido*-2-*acetoxybenzoate* (3·2 g.) separated as needles, m. p. 145—147°; it does not give a coloration with aqueous ferric chloride solution (Found: C, 57·8; H, 5·7; N, 5·5. $C_{12}H_{13}O_5N$ requires C, 57·4; H, 5·2; N, 5·6%).

A solution of methyl 4-acetamido-2-acetoxybenzoate (0·5 g.) in methanol (20 c.c.) was treated with one of potassium carbonate (0·5 g.) in water (2 c.c.) and the mixture kept at room temperature for 60 hours. The mixture was evaporated (reduced pressure) and the residue extracted with ether. Evaporation of the ethereal solution and crystallisation of the residue from aqueous methanol gave methyl 4-acetamidosalicylate as needles, m. p. 150°, undepressed when mixed with the specimen above.

Methyl 4-*benzamidosalicylate*, prepared by treatment of 4-benzamidosalicylic acid with diazomethane in ether–methanol, separates from benzene as needles, m. p. 168° (Found: C, 65·9; H, 4·9; N, 5·3. $C_{15}H_{13}O_4N$ requires C, 66·4; H, 4·8; N, 5·2%).

Methyl 4-*benzamido*-2-*benzoyloxybenzoate* was obtained by benzoylation of methyl 4-aminosalicylate or of methyl 4-benzamidosalicylate by means of benzoyl chloride and sodium hydroxide solution. It separates from aqueous ethanol as needles, m. p. 158—159° (Found: C, 69·8; H, 4·7; N, 3·4. $C_{22}H_{17}O_5N$ requires C, 70·4; H, 4·5; N, 3·7%).

Methyl 4-*Acetylsulphanilamidosalicylate*.—A solution of methyl 4-aminosalicylate (3·36 g) in dry pyridine (25 c.c) was treated with acetylsulphanilyl chloride (5·12 g). The solution was heated on a steam-bath for 90 minutes and poured on crushed ice (100 g.). The solid was collected and crystallised from aqueous alcohol, from which *methyl* 4-*acetylsulphanilamidosalicylate* (3·2 g.) separated as plates, m. p. 238° (Found: C, 52·3; H, 4·5; N, 7·5. $C_{16}H_{16}O_6N_2S$ requires C, 52·7; H, 4·4; N, 7·7%).

Methyl 4-*Sulphanilamidosalicylate*.—A solution of the acetyl compound (1·6 g.) in 75% ethanol (10 c.c.) was heated under reflux with 10% hydrochloric acid (5 c.c.) for 2 hours. The solution was evaporated under reduced pressure, and the residue crystallised from aqueous ethanol to yield *methyl* 4-*sulphanilamidosalicylate* (1·2 g.) as plates, m. p. 210° (Found: C, 52·5; H, 4·4; N, 9·1. $C_{14}H_{14}O_5N_2S$ requires C, 52·2; H, 4·3; N, 8·7%), insoluble in sodium hydrogen carbonate solution.

4-*Sulphanilamidosalicylic Acid*.—A solution of the methyl ester (1·2 g.) in excess of alcoholic potassium hydroxide solution (10%) was heated under reflux for 2 hours. The mixture was adjusted to pH 4·0 by addition of hydrochloric acid. The solid was collected and crystallised from aqueous ethanol, from which 4-*sulphanilamidosalicylic acid* (0·7 g.) separates as prisms, m. p. 220° (decomp.) (Found: C, 51·1; H, 4·2; N, 8·8. $C_{13}H_{12}O_5N_2S$ requires C, 50·65; H, 3·9; N, 9·1%). It is soluble in cold sodium hydrogen carbonate solution with effervescence. The same acid was also obtained by treatment of 4-aminosalicylic acid with acetylsulphanilyl chloride, followed by hydrolysis of the product (Found: C, 50·8; H, 4·2; N, 8·9; S, 10·6%).

2-*Nitro*-4-*hydroxybenzoic Acid*.—A warm solution of 2-nitro-4-aminobenzoic acid (18·2 g.) in water (30 c.c.) and concentrated sulphuric acid (22 c.c.) was rapidly chilled to 0°. After the addition of crushed ice (50 g.), a solution of sodium nitrite (7·5 g.) in water (16 c.c.) was added during 1 hour at 0—5°. The mixture was stirred for a further hour at this temperature, treated with urea (10 g.), and filtered. The solution was slowly added (1 hour) to a boiling solution of sulphuric acid (70 c.c., conc.) in water (50 c.c.), and the mixture heated under reflux for 3 hours. On cooling, 2-*nitro*-4-*hydroxybenzoic acid* (7·5 g.) separated as light yellow plates, m. p. 230° (decomp.) (Found: C, 46·1; H, 3·0; N, 7·4. $C_7H_5O_5N$ requires C, 45·9; H, 2·7; N, 7·65%).

Methyl 2-*nitro*-4-*hydroxybenzoate* was obtained by heating a solution of the acid (3·7 g.) with methanol (80 c.c.) containing concentrated sulphuric acid (2 c.c.) under reflux for 18 hours. It separated from water as needles, m. p. 168° (Found: C, 49·0; H, 3·5; N, 6·9. $C_8H_7O_5N$ requires C, 48·7; H, 3·55; N, 7·1%).

Ethyl 2-*nitro*-4-*hydroxybenzoate*, prepared in a similar manner, separates from water as needles, m. p. 157° (Found: C, 51·3; H, 4·2; N, 6·5. $C_9H_9O_5N$ requires C, 51·2; H, 4·3; N, 6·6%).

4-*Hydroxyanthranilic Acid*.—A solution of 2-nitro-4-hydroxybenzoic acid (4·6 g.) in ethyl alcohol

(100 c.c.) was shaken with hydrogen and platinum (from platinum oxide) at room temperature and pressure, until hydrogen absorption was complete. The filtered solution was evaporated, and the solid recrystallised from water to give *4-hydroxyanthranilic acid* (3 g.) as prismatic needles, m. p. 158° (decomp.). The m. p. varied with the rate of heating (Found: C, 55·2; H, 4·8; N, 9·4. $C_7H_7O_3N$ requires C, 54·9; H, 4·5; N, 9·15%). The *hydrochloride* crystallised from concentrated hydrochloric acid in needles (Found: C, 44·1; H, 4·3; N, 7·4. $C_7H_8O_3NCl$ requires C, 44·3; H, 4·2; N, 7·4%). It decomposes at 155° and finally melts above 220°, a behaviour which may indicate preliminary decomposition to 3-aminophenol hydrochloride (m. p. 237°).

Methyl 4-hydroxyanthranilate, obtained by similar catalytic reduction of methyl 2-nitro-4-hydroxybenzoate, separates as needles from water, m. p. 134·5—135° (Found: C, 57·9; H, 5·5; N, 8·25. $C_8H_9O_3N$ requires C, 57·5; H, 5·4; N, 8·4%). A mixture with methyl 4-aminosalicylate (m. p. 119—120°) melted at 100—104°.

Ethyl 4-hydroxyanthranilate separates as needles from water, m. p. 119—120°. A mixture with ethyl 4-aminosalicylate (m. p. 110—112°) has m. p. 95—100° (Found: C, 59·7; H, 6·1; N, 7·6. $C_9H_{11}O_3N$ requires C, 59·7; H, 6·1; N, 7·7%).

Our thanks are due to Mr. C. W. Picard, M.Sc., who collaborated in the early stages of this work, and to Mr. J. Lowenthal, B.Sc., for considerable assistance in the experimental work.

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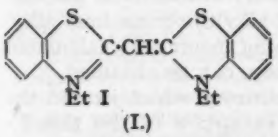
319. *The Reactivity of the Alkylthio-group in Nitrogen Ring Compounds. Part I. A General Method for the Preparation of Symmetrical and Unsymmetrical Thiacyanines.*

By J. D. KENDALL and H. G. SUGGATE.

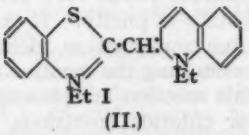
The preparation is described of symmetrical and unsymmetrical monomethin thiacyanines by the reaction, in the presence of basic reagents, of 2-alkylthiothiazolium alkyl salts with quaternary heterocyclic ammonium salts containing a reactive methyl group.

THE first symmetrical thiacyanine, 3 : 3′-diethylthiacyanine iodide (I), was isolated by Mills (*J.*, 1922, **121**, 458) from the products of the action of ammonia on an aqueous solution of benzthiazole ethiodide and 2-methylbenzthiazole ethiodide, and its constitution established by synthesis from *o*-aminothiophenol and diethyl malonate. Subsequently, Mills and Braunholtz (*J.*, 1923, 2804) showed that thiacyanines could also be obtained in low yield, in admixture with thiacarbocyanines, by interaction of 2 : 2′-di-(*N*-formyl-*N*-ethylamino)diphenyl disulphide and a 2-methylbenzthiazolium alkyl salt in pyridine. By the action of amyl nitrite and acetic anhydride on a 2-methylbenzthiazolium alkyl salt, selectively a chloride, Fisher and Hamer (*J.*, 1930, 2502) prepared a number of symmetrical thiacyanines, free from the thiacarbocyanines always formed simultaneously by other methods. Their general method, however, was of limited scope and failed with the alkyl salts of simple thiazoles and 2-methyl-β-naphthothiazole.

Using the well-known *pseudo*cyanine and *iso*cyanine condensations, a number of unsymmetrical thiacyanines were also prepared by Mills and Braunholtz (*loc. cit.*), Hamer (*J.*, 1928, 206), Hamer and Kelly (*J.*, 1931, 777), Fisher and Hamer (*J.*, 1934, 1905). These thiacyanines, *e.g.*, 3 : 1′-diethylthia*pseudo*cyanine iodide (II) and 3 : 1′-diethylthia*iso*cyanine iodide (III), contained as second heterocyclic nucleus a pyridine or substituted pyridine ring.



(I.)



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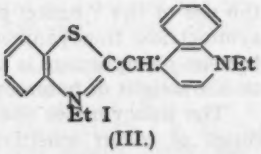


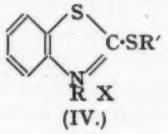
(III.)

Attempts by the present authors to obtain symmetrical and unsymmetrical thiacyanines by replacing, with 2-iodothiazolium alkiodides, the 2-iodoquinoline alkiodides used in the *pseudo*cyanine condensation, were unsuccessful. Although both 2-chloro-4-methylthiazole and 2-chlorobenzthiazole react readily on heating with alkyl iodides to give what are apparently 2-iodothiazolium alkiodides, these salts have low solubility in all solvents and, unlike 2-iodoquinoline alkiodides, do not form cyanine dyes with quaternary heterocyclic ammonium salts containing a reactive methyl group. By interaction, however, in the presence of a basic reagent,

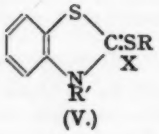
of the readily accessible 2-alkylthiothiazolium alkyl salts (IV) with heterocyclic ammonium salts containing a reactive methyl group, both symmetrical and unsymmetrical thiacyanines (VI) are readily prepared. This method was discovered independently by I. G. Farbenindustrie A.G. (B.P. 423,492) and one of the present authors (Kendall, B.P. 424,559). I. G. Farbenindustrie, however, did not envisage the use of an alkyl salt of 2-alkylthiobenzthiazole, but of a 2-thio-3-alkyl-2 : 3-dihydrobenzthiazole alkyl salt which they regarded as a sulphonium compound (V). We prefer to regard as quaternary heterocyclic ammonium salts the compounds (IV) and (V), which are identical, prepared by either process, when R = R′.

Unlike 2-alkoxybenzthiazole and 2-keto-3-alkyl-2 : 3-dihydrobenzthiazoles which do not form stable salts with alkyl iodides, 2-alkylthiobenzthiazoles and 2-thio-3-alkyl-2 : 3-dihydrobenzthiazoles form, with methyl and ethyl iodide, salts which can be readily isolated. Contrary to the result obtained by Beilenson and Hamer (*J.*, 1939, 143) who recorded no group interchange, Sexton (*J.*, 1939, 470) states that the reaction of ethyl iodide with 2-methylthiobenzthiazole gives 2-ethylthiobenzthiazole methiodide and not 2-methylthiobenzthiazole ethiodide as expected. Experiments carried out in this laboratory, and discussed in another communication, indicate that a mixture of quaternary salts is formed. The salts obtained by interaction of 2-alkylthiobenzthiazoles and alkyl toluene-*p*-sulphonates also appear in certain cases to undergo anomalous quaternisations. The addition of an alkyl iodide to a thione produces one compound only.

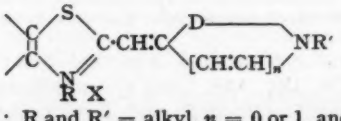
The alkyl salts of 2-alkylthiobenzthiazoles are readily converted into thiones by heat, or by a solvent containing a basic reagent (Kendall, B.P. 475,647). Crystallisation from alcoholic solvents likewise effects decomposition, which can be detected by the odour of alkanethiol in the solvent. Because of the ease with which they are formed, the thiones are found as the principal by-products when 2-alkylthiothiazolium alkyl salts are used in cyanine dye condensations; they can be removed easily from the crude dyes by washing with hot benzene in which the dyes are insoluble.



(IV.)



(V.)



(VI : R and R′ = alkyl, *n* = 0 or 1, and D is remainder of a heterocyclic ring.)

The tendency to thione formation can be reduced, and the yields of thiacyanines increased, by the use of the " fusion process " (Kendall, B.P. 438,420), in which an alkyl toluene-*p*-sulphonate, a heterocyclic base with a reactive methyl group, and a 2-alkylthiobenzthiazole are heated together to at least 120°. Under these conditions partial formation of dye occurs during the fusion, and the condensation can be completed by dissolution in a solvent which may be a base, *e.g.*, pyridine, or which contains a basic reagent, *e.g.*, ethanol to which sodium ethoxide, sodium acetate, or an alkylamine is added. For the preparation of unsymmetrical thiacyanines with two different alkyl groups attached to the nitrogen of the two heterocyclic rings, it is essential in the " fusion process " to use a thione with the required alkyl group attached to the nitrogen. The dye toluene-*p*-sulphonates can be converted into less soluble halide salts by addition of a solution of the former to an aqueous alkali halide solution containing a considerable excess of the halide. In a number of cases in which only a slight excess of the halide was used the resulting dye still contained unchanged toluenesulphonate. By the use of the " fusion process " excellent yields were obtained of many symmetrical and unsymmetrical thiacyanines in a high state of purity. If excess of a 2-alkylthiothiazolium alkyl toluene-*p*-sulphonate is used in the dye condensation, yields approaching theoretical, calculated on the weight of heterocyclic base containing the reactive methyl group, can be obtained.

The thiacyanines obtained by this reaction are photographic sensitizers, which extend the range of colour sensitivity of silver chloride emulsions, with the exception of the thia-2′-quinocyanine (II) and thia-4′-quinocyanine (III) which also sensitise silver iodobromide emulsions.

A few thiacyanines, prepared by our process, have been described by Beilenson and Hamer (*loc. cit.*).

Experimental.

2-Iodo-4-methylthiazole Ethiodide.—2-Chloro-4-methylthiazole (30 g.) and ethyl iodide (40 c.c.) were heated under reflux on a water-bath for 48 hours. The dark-brown crystals which separated were filtered off and washed well with ethanol and ether; they (16·9 g.) were almost insoluble in the usual

solvents and were purified by boiling out with acetone, and then had m. p. 171° (decomp.) (Found: I, 66·1. $C_8H_9NI_2S$ requires I, 66·7%).

2-Iodobenzthiazole Methiodide.—This salt, prepared similarly from 2-chlorobenzthiazole (B.P. 310,815) (10 g.) and methyl iodide (10 c.c.), was obtained as brown crystals (16 g.), m. p. 203° (decomp.), by boiling out with acetone (Found: I, 63·3. $C_8H_7NI_2S$ requires I, 63·0%). *2-Iodobenzthiazole ethiodide*, similarly prepared (1·3 g. from 8 g. of each reagent) and purified by boiling out with acetone, had m. p. 188° (decomp.) (Found: I, 60·7. $C_9H_9NI_2S$ requires I, 60·9%).

2-Methoxybenzthiazole.—A solution of sodium (5 g.) in methanol (100 c.c.) was added to a solution of 2-chlorobenzthiazole (33·8 g.) in methanol (100 c.c.), and the mixture boiled for 2 hours. Excess of alcohol was distilled off, the residue diluted with water (500 c.c.), and the precipitated oil separated and dried (K_2CO_3). On distillation (b. p. 135—137°/16 mm.) the product was obtained as a colourless oil (25 g.) which solidified on cooling. Recrystallisation from ethanol (2 c.c. per g.) gave colourless rhombs, m. p. 34° (Found: S, 19·6. Calc. for C_8H_7ONS: S, 19·4%).

3 : 4-Dimethyl-1 : 2-dihydrothiazole-2-thione.—Monochloroacetone (10·4 c.c.) was added gradually with cooling to a suspension of ammonium methyldithiocarbamate (18 g.) in ethanol (36 c.c.). Addition of water precipitated a solid which after several crystallisations from a 50% ethanol–water mixture (1 g. in 10 c.c.) was obtained as pale yellow crystals (4·4 g.), m. p. 119° (Found: S, 44·1. $C_5H_7NS_2$ requires S, 44·1%).

3-Phenyl-4-methyl-1 : 2-dihydrothiazole-2-thione.—Ammonium phenyldithiocarbamate (93 g.) was suspended in ethanol (250 c.c.), and monochloroacetone (47 c.c.) added in portions with cooling. The mixture, from which ammonium chloride had separated, was boiled for 30 minutes and diluted with water (500 c.c.), and the crystalline precipitate collected and recrystallised from ethanol (1 g. in 10 c.c.). It was obtained as pale buff crystals (63 g.), m. p. 148—149° (Found: S, 31·1. $C_{10}H_9NS_2$ requires S, 30·9%).

2-Methylthiobenzthiazole.—Commercial 2-mercaptobenzthiazole (400 g.) was dissolved in 10% sodium hydroxide solution (1 l.). The solution was filtered, and methyl sulphate (230 c.c.) added with stirring (1 hour), the temperature being kept at about 40° by cooling. The precipitated oil was extracted with ether and dried (K_2CO_3), and the solvent removed. Distillation of the residue gave the product as an almost colourless oil (326 g.) which solidified on cooling; b. p. 174—175°/22 mm., m. p. 46° (Found: S, 35·2. Calc. for $C_8H_7NS_2$: S, 35·4%). If the methyl sulphate was added too rapidly, or in excess, the yield of 2-methylthiobenzthiazole was lowered, appreciable quantities of 1-methyl-2 : 3-dihydrobenzthiazole-2-thione being obtained as a by-product.

2-Ethylthiobenzthiazole.—2-Mercaptobenzthiazole (200 g.) was boiled under reflux with ethanol (500 c.c.) and 20% sodium hydroxide solution (300 c.c.). Ethyl bromide (100 c.c.) was then added gradually (1 hour) and the mixture heated for a further hour. After the solution had cooled, the precipitated oil was removed, dissolved in ether, washed with water, and dried (K_2CO_3). Distillation gave a pale yellow oil (170 g.), b. p. 178°/18 mm., m. p. 26° (Found: S, 33·0. Calc. for $C_9H_9NS_2$: S, 32·9%).

2-Mercapto-β-naphthothiazole.—Carbon disulphide (87 c.c.) and ammonia solution (*d* 0·920; 180 c.c.) were mixed at 0°, and α-naphthylamine (176 g.) in ethanol (350 c.c.) added dropwise with stirring, the temperature being kept below 10°. The mixture was kept overnight at room temperature, and ethyl chloroformate (130 c.c.) then added slowly with stirring, the temperature being kept below 5°. The temperature of the mixture was then allowed to rise to 20°, water (2·5 l.) added, and the precipitated oil extracted with ether, dried (K_2CO_3), and distilled to give α-naphthyl *iso*thiocyanate as a white, waxy solid (208 g.), b. p. 190°/20 mm. m. p. 57—58°. An intimate mixture of α-naphthyl *iso*thiocyanate (88 g.) and sulphur (16 g.) was heated in a sealed tube for 5 hours at 240—250°. The brown amorphous product was extracted four times with 5% sodium hydroxide solution. The alkaline solution, on acidification with acetic acid, gave pale yellow rhombs (29 g.) which were crystallised from ethanol; m. p. 254°.

2-Methylthio-β-naphthothiazole.—2-Mercapto-β-naphthothiazole (70 g.) was dissolved in 5% sodium hydroxide solution (260 c.c.), and methyl sulphate (35 c.c.) added slowly with stirring, the temperature being kept below 45°. After one hour the precipitated oil was separated, dissolved in benzene, and dried (K_2CO_3), and the solvent removed. The residue was distilled (b. p. 246—248°/20 mm.) to give a yellow solid which on recrystallisation from ethanol formed pale yellow crystals (62 g.), m. p. 63° (Found: S, 27·6. Calc. for $C_{12}H_9NS_2$: S, 27·7%).

2-Ethylthio-β-naphthothiazole.—2-Mercapto-β-naphthothiazole (75 g.) was heated to boiling with a mixture of ethanol (128 c.c.) and 20% sodium hydroxide solution (91·5 c.c.). Ethyl bromide (46 c.c.) was run in slowly, the mixture being kept at boiling point during addition. After further heating of the mixture for 2 hours, water (2 l.) was added, and the precipitated oil separated and dried (K_2CO_3). Distillation yielded the product as a pale yellow oil (48·5 g.), b. p. 248—250°/18 mm. (Found: S, 25·8. Calc. for $C_{13}H_{11}NS_2$: S, 26·1%).

2-Methylthio-α-naphthothiazole.—2-Mercapto-α-naphthothiazole (200 g.) was dissolved in 2½% sodium hydroxide solution (1·6 l.), and methyl sulphate (80 c.c.) added slowly with stirring. After two hours the precipitated solid was filtered off, dried, and recrystallised from ethanol (50 c.c. per g.) to give pale yellow crystals (170 g.), m. p. 67—68° (Found: S, 27·5. Calc. for $C_{12}H_9NS_2$: S, 27·7%).

2-Ethylthio-α-naphthothiazole.—2-Mercapto-α-naphthothiazole (150 g.) was heated under reflux with ethanol (450 c.c.), and 20% sodium hydroxide solution (184 c.c.) and ethyl bromide (96 c.c.) were added slowly. The mixture was boiled for 2 hours and then cooled, and water (3 l.) was added. The product formed cream-coloured crystals (146 g.), m. p. 49°, b. p. 248—250°/18 mm. from ethanol (50 c.c. per g.) (Found: S, 25·9. Calc. for $C_{13}H_{11}NS_2$: S, 26·1%).

2-Mercapto-5 : 6-dimethoxybenzthiazole.—4-Bromo-5-nitroveratrole (230 g.) (Jones and Robinson, *J.*, 1917, **111**, 916) was suspended in boiling ethanol (600 c.c.), and sodium hydrogen sulphide (365 g.) in cold ethanol (365 c.c.) added slowly. The mixture was heated under reflux for 1 hour and then cooled; hydrogen sulphide was passed in during one hour. Carbon disulphide (106 c.c.) was added, and the brown mixture was then boiled for seven hours, and cooled, whereupon the sodium salt of the thiol,

with some sodium bromide, was precipitated (302 g.). Addition of hydrochloric acid to a solution of the crude sodium salt gave 2-*mercapto*-5 : 6-*dimethoxybenzthiazole* which crystallised from ethanol as pale rhombs, m. p. 209° (Found : S, 27·8. $C_9H_9O_2NS_2$ requires S, 28·2%).

2-*Methylthio*-5 : 6-*dimethoxybenzthiazole*.—The above-mentioned crude sodium salt (200 g.) was dissolved in hot water (1 l.) and cooled to 85°, and methyl sulphate (55 c.c.) run in with stirring. 10% aqueous sodium hydroxide (100 c.c.) was added to decompose the excess of methyl sulphate, and the precipitated *methylthio*-compound filtered off and crystallised from ethanol (200 c.c.) to give almost colourless rhombs (92 g.), m. p. 115° (Found : S, 26·4. $C_{10}H_{11}O_2NS_2$ requires S, 26·5%).

2-*Ethylthio*-5 : 6-*dimethoxybenzthiazole*.—The crude sodium salt (92 g.) was suspended in ethanol (100 c.c.), ethyl bromide (71 c.c.) added gradually, and the solution boiled under reflux for 6 hours. Excess ethyl bromide and ethanol were distilled off, the residue diluted with water (2 l.), and the precipitated oil extracted with ether. The extracts were washed with water, and dried (K_2CO_3), and the solvent removed. The residual oil was distilled (b. p. 235—242°/12 mm.), and the *product* crystallised from ethanol to give colourless needles (29·1 g.), m. p. 63° (Found : S, 25·3. $C_{11}H_{13}O_2NS_2$ requires S, 25·10%).

2-*Mercapto*-5 : 6-*methylenedioxybenzthiazole*.—This was prepared as described for the 5 : 6-dimethoxy-analogue, from 4-bromo-5-nitropyrocatechol methylene ether (242 g.) (Jones and Robinson, *ibid.*, p. 919). 2-*Mercapto*-5 : 6-*methylenedioxybenzthiazole* crystallised from ethanol as buff-coloured rhombs, m. p. 263° (Found : S, 30·9. $C_8H_5O_2NS_2$ requires S, 30·3%).

2-*Methylthio*-5 : 6-*methylenedioxybenzthiazole*.—The crude sodium salt (160 g.) of 2-mercapto-5 : 6-methylenedioxybenzthiazole was dissolved in water (1·8 l.) and filtered from a little insoluble material. Methyl sulphate (62 c.c.) was added with stirring, the temperature being kept at about 30°. The *methylthio*-compound separated during the reaction as dull yellow crystals, which were filtered off after addition of aqueous sodium hydroxide (100 c.c. of 10% solution). Recrystallisation from ethanol, containing a little norite, gave the product as pale needles (64 g.), m. p. 160° (Found : S, 28·5. $C_9H_7O_2NS_2$ requires S, 28·5%).

2-*Ethylthio*-5 : 6-*methylenedioxybenzthiazole*.—The crude sodium salt of 2-mercapto-5 : 6-methylenedioxybenzthiazole (60 g.) was suspended in dry ethanol (700 c.c.) ethyl bromide (43 c.c.) added gradually, and the mixture boiled under reflux for 8 hours and filtered hot buff-coloured plates. Recrystallisation of the *product* which separated after cooling, from ethanol (1 g. in 5 c.c.) gave very pale-buff crystals (27 g.), m. p. 127—128° (Found : S, 26·8. $C_{10}H_9O_2NS_2$ requires S, 26·8%).

5 : 6-*Dimethoxy*-2-*methylbenzthiazole*.—4-Bromo-5-nitroveratrole (193 g.) was suspended in ethanol (1 l.) and treated gradually, with shaking, with a solution of sodium disulphide prepared from crystalline sodium sulphide (94 g.), sulphur (13·1 g.), and ethanol (120 c.c.). Addition of the disulphide solution caused a violent reaction, with dissolution of the bromonitroveratrole and precipitation of dark crystals which became bright yellow after the mixture had been boiled for 1½ hours. The precipitated 2 : 2′-dinitro-4 : 5 : 4′ : 5′-tetramethoxydiphenyl disulphide (153 g.) was filtered off after cooling, washed with hot water and then with ethanol, and dried; m. p. 219°. The crude disulphide was suspended in glacial acetic acid (1 l.) and acetic anhydride (565 c.c.) and reduced with zinc dust (320 g.). The mixture was filtered, excess of acetic acid and anhydride distilled off, and the residue distilled under reduced pressure; b. p. 188°/16 mm. The *product* formed buff-coloured needles, m. p. 65°, from ethanol (Found : S, 14·9. $C_{10}H_{11}O_2NS$ requires S, 15·3%).

5 : 6-*Methylenedioxy*-2-*methylbenzthiazole*.—4-Bromo-5-nitrocatechol methylene ether (125 g.) was treated, in ethanol (190 c.c.), gradually with frequent shaking, with a sodium disulphide solution prepared from crystalline sodium sulphide (62 g.), sulphur (9 g.), and ethanol (80 c.c.) (cf. above). A suspension of the crude 2 : 2′-dinitro-4 : 5-4′ : 5′-bis(methylenedioxy)diphenyl disulphide (m. p. 244°) (92 g.) thus produced, in glacial acetic acid (449 c.c.) and acetic anhydride (280 c.c.), was warmed to 80° and commercial zinc dust (151 g.) added in small portions. Working up as described above gave 5 : 6-*methylenedioxy*-2-*methylbenzthiazole* as colourless plates, m. p. 123—124° (from ethanol), b. p. 195—200°/20 mm. (Found : S, 16·9. $C_9H_7O_2NS$ requires S, 16·6%).

[3 : 4-*Dimethyloxazole*][3-*methylbenzthiazole*]*methincyanine Iodide*.—2 : 4-Dimethyloxazole (3 g.), 2-methylthiobenzthiazole (5·6 g.), and methyl toluene-*p*-sulphonate (11·5 g.) were fused together at 130—140° for three hours. Pyridine (20 c.c.) was added and the mixture boiled under reflux for 30 minutes. On pouring this into a warm aqueous solution of potassium iodide (8 g. in 50 c.c.), the *iodide* was precipitated; it crystallised from methanol as pale yellow prismatic needles (2 g.), m. p. 309° (decomp.) (Found : I, 33·0. $C_{14}H_{15}ON_2IS$ requires I, 32·9%).

[3 : 4-*Dimethylthiazole*][3-*methylbenzthiazole*]*methincyanine Bromide*.—This *bromide* was prepared by a process similar to that used for bis[3-methylbenzthiazole]methincyanine bromide, but from 2 : 4-dimethylthiazole (2·26 g.), 2-methylthiobenzthiazole (3·62 g.), and methyl toluene-*p*-sulphonate (7·44 g.). The dye was obtained from methanol (1 g. in 50 c.c.) as yellow rods and needles (2·7 g.), m. p. 298° (decomp.) (Found : Br, 22·7. $C_{14}H_{15}N_2S_2Br$ requires Br, 22·6%). It was also obtained from 3 : 4-dimethyl-1 : 2-dihydrothiazole-2-thione (1·44 g.), 2-methylbenzthiazole (1·49 g.), and methyl toluene-*p*-sulphonate (3·72 g.).

The following were similarly prepared. [4-*Methyl*-3-*ethylthiazole*][3-*ethylbenzthiazole*]*methincyanine iodide* [from 2 : 4-dimethylthiazole (2·26 g.), 2-ethylthiobenzthiazole (3·9 g.), and ethyl toluene-*p*-sulphonate (8 g.)], yellow prisms (1·8 g.), m. p. 297° (decomp.) [from methanol (1 g. in 200 c.c.)] (Found : I, 29·5. $C_{16}H_{19}N_2IS_2$ requires I, 29·6%). [3 : 4-*Dimethylthiazole*][1 : 6-*dimethyl*-2-*quinoline*]*methincyanine iodide* [from 2-methylthio-4-methylthiazole (Buckman, Reims, and Sargent, *J. Org. Chem.*, 1941, **6**, 764) (1·8 g.), *p*-toluquinaldine (1·95 g.), and methyl toluene-*p*-sulphonate (4·7 g.)], orange-red (1·5 g.), m. p. 238° (decomp.) [from methanol (1 g. in 50 c.c.)] (Found : I, 31·1. $C_{17}H_{19}N_2IS$ requires I, 30·9%). [3-*Phenyl*-4-*methylthiazole*][1-*methyl*-2-*quinoline*]*methincyanine iodide* [from quinaldine (1·43 g.), 3-phenyl-4-methyl-1 : 2-dihydrothiazole-2-thione (2·07 g.), and methyl toluene-*p*-sulphonate (3·72 g.)], reddish-brown (1 g.), m. p. 230° (decomp.) [from methanol (1 g. in 30 c.c.)] (Found : I, 27·6. $C_{21}H_{19}N_2IS$ requires I, 27·7%).

[3-*Phenyl*-4-*methylthiazole*][3-*methylbenzthiazole*]*methincyanine Iodide*.—3-Phenyl-4-methyl-1 : 2-di-

hydrothiazole-2-thione (2·07 g.), 2-methylbenzthiazole (1·49 g.), and methyl toluene-*p*-sulphonate (3·72 g.) were fused together at 150° for 4 hours; pyridine (25 c.c.) was added and the mixture boiled under reflux for 40 minutes; the yellow solution was poured into an aqueous solution of potassium iodide (5 g.), and the *dye* collected and washed with hot benzene, ethanol, and ether. It crystallised from methanol (1 g. in 10 c.c.) as pale yellow needles (2·9 g.), m. p. 264° (decomp.) (Found: I, 27·3. $C_{19}H_{17}N_2IS_2$ requires I, 27·4%).

The following were similarly prepared. [3-*Methylbenzoxazole*][3-*methylbenzthiazole*]*methincyanine iodide* [from 2-methylbenzoxazole (1·33 g.), 2-methylthiobenzthiazole (1·81 g.), and methyl toluene-*p*-sulphonate (3·72 g.)], pale-yellow rhombs (2·96 g.), m. p. 310° (decomp.) [from methanol (1 g. in 200 c.c.)] (Found: I, 30·3. $C_{17}H_{15}ON_2IS$ requires I, 30·1%). [3-*Ethylbenzoxazole*][3-*ethylbenzthiazole*]-*methincyanine iodide* [from 2-ethylthiobenzthiazole (1·95 g.), 1-methylbenzoxazole (1·33 g.), and ethyl toluene-*p*-sulphonate (4 g.)], yellow needles (2·27 g.), m. p. 309° (decomp.) [from methanol (1 g. in 200 c.c.)] (Found: I, 28·5. $C_{19}H_{19}ON_2IS$ requires I, 28·2%). [3-*Ethylnaphtho*(1′ : 2′-4 : 5)*oxazole*]-[3-*ethylbenzthiazole*]*methincyanine iodide* [from 2-ethylthiobenzthiazole (0·98 g.), 2-methylbenzoxazole (0·92 g.), and ethyl toluene-*p*-sulphonate (2·0 g.)], small straw-coloured needles (0·8 g.), m. p. 283° (decomp.) [from methanol (1 g. in 50 c.c.)] (Found: I, 25·4. $C_{23}H_{21}ON_2IS$ requires I, 25·4%). [3-*Ethylnaphtho*(2′ : 1′-4 : 5)*oxazole*][3-*ethylbenzthiazole*]*methincyanine iodide* [from 2-methylnaphtho(2′ : 1′-4 : 5)oxazole (3·6 g.), 2-methylthiobenzthiazole (3·8 g.), and ethyl toluene-*p*-sulphonate (8·3 g.)], bright yellow needles (0·6 g.), m. p. 298° (decomp.) [from methanol (1 g. in 400 c.c.)] (Found: I, 25·4. $C_{23}H_{21}ON_2IS$ requires I, 25·4%).

Bis-[3-*methylbenzthiazole*]*methincyanine Bromide.*—2-Methylthiobenzthiazole (3·1 g.), 2-methylbenzthiazole (2·5 g.), and methyl toluene-*p*-sulphonate (6·5 g.) were fused together at 140° for 2½ hours. Pyridine (30 c.c.) was added, and the mixture boiled gently for 45 minutes. A bright yellow solution formed and methylthiol was evolved. On pouring the solution into a warm aqueous solution of potassium bromide (10 g. in 100 c.c.), a yellow precipitate was obtained. This was filtered off, washed with water, hot benzene, and ether, and crystallised from methanol. The dye *bromide* was obtained as light yellow needles (4·5 g.), m. p. 303° (decomp.) (Found: Br, 20·7. $C_{17}H_{15}N_2BrS_2$ requires Br, 20·5%).

A small quantity of the dye iodide was obtained by pouring the original aqueous filtrate into a solution of potassium iodide (10 g. in 100 c.c.). The yellow precipitate crystallised from methanol in minute yellow needles, m. p. 306° (decomp.).

[3-*Ethylbenzthiazole*][3-*methylbenzthiazole*]*methincyanine Bromide.*—2-Methylthiobenzthiazole (1·81 g.) and methyl toluene-*p*-sulphonate (1·86 g.) were fused together for 3½ hours at 130°. A similar fusion was also carried out with 2-methylbenzthiazole (1·49 g.) and ethyl toluene-*p*-sulphonate (2·0 g.). The ethotoluene-*p*-sulphonate was then dissolved in pyridine (10 c.c.), and the solution poured into the 2-methylthiobenzthiazole methotoluene-*p*-sulphonate. The mixture was boiled for 45 minutes and then poured into potassium bromide (10 g.) in water (100 c.c.); and the yellow *bromide* was filtered off, washed with water, hot benzene, and ether, and crystallised from methanol; pale-yellow needles (2·5 g.), m. p. 283° (decomp.) (Found: Br, 19·9. $C_{19}H_{17}N_2BrS_2$ requires Br, 19·8%).

Bis-[3-*ethylbenzthiazole*]*methincyanine Bromide.*—This dye, prepared as described for the bis-3-methyl analogue, crystallised from methanol (40 c.c. per g.) as yellow needles (9·3 g.), m. p. 303° (decomp.). The derived yellow dye iodide crystallised from methanol (100 c.c. per g.); m. p. 314° (decomp.) (Found: I, 27·1. Calc. for $C_{19}H_{19}N_2IS_2$: I, 27·3%).

[3-*Methylbenzthiazole*][3-*methyl*-β-*naphthothiazole*]*methincyanine Bromide.*—2-Methyl-β-naphthothiazole with methyl toluene-*p*-sulphonate (3·7 g.), and 2-methylthiobenzthiazole (3·62 g.) with methyl *p*-toluenesulphonate (3·72 g.), similarly yielded a *dye*, yellow needles (5·6 g.), m. p. 250° (decomp.), from methanol (1 g. in 200 c.c.) (Found: Br, 17·9. $C_{21}H_{17}N_2BrS_2$ requires Br, 18·2%).

[3-*Ethylbenzthiazole*][3-*ethyl*-β-*naphthothiazole*]*methincyanine Bromide.*—2-Methyl-β-naphthothiazole (4 g.) and ethyl toluene-*p*-sulphonate (4 g.) were fused together for 3½ hours at 190°. A similar fusion was carried out with 2-ethylthiobenzthiazole (3·9 g.) and ethyl toluene-*p*-sulphonate (4 g.) at 140° for 3 hours. The 2-ethylthiobenzthiazole ethotoluene-*p*-sulphonate was cooled and dissolved in pyridine (30 c.c.), the solution poured on the 2-methyl-β-naphthothiazole salt, and the mixture heated under reflux for 45 minutes. On pouring the product into a warm aqueous solution of potassium bromide (10 g. in 100 c.c.), yellow crystals were obtained which were filtered off, washed with hot benzene, water, and ether, and recrystallised from methanol. The *product* consisted of yellow rhombs (2 g.), m. p. 252° (decomp.) (Found: Br, 16·8. $C_{23}H_{21}N_2BrS_2$ requires Br, 17·1%).

Similar preparations yielded: [3-*methylbenzthiazole*][3-*methyl*-5 : 6-*dimethoxybenzthiazole*]*methincyanine toluene*-p-*sulphonate*, from 5 : 6-dimethoxy-2-methylbenzthiazole (2·09 g.), 2-methylthiobenzthiazole (1·81 g.), and methyl toluene-*p*-sulphonate (37 g.), as yellow needles (3·1 g.), m. p. 315° (decomp.), from methanol (1 g. in 180 c.c.) (Found: S, 17·4, 17·5. $C_{26}H_{26}O_2N_2S_2$ requires S, 17·7%); [3-*ethylbenzthiazole*][5 : 6-*dimethoxy*-3-*ethylbenzthiazole*]*methincyanine bromide*, from 5 : 6-dimethoxy-2-methylbenzthiazole (1·2 g.), 2-ethylthiobenzthiazole (0·98 g.), and ethyl toluene-*p*-sulphonate (2 g.), as yellow rhombs (0·5 g.), m. p. 271° (decomp.), from methanol (1 g. in 100 c.c.) (Found: Br, 16·6. $C_{21}H_{23}O_2N_2BrS_2$ requires Br, 16·6%); [3-*methylbenzthiazole*][5 : 6-*methylenedioxy*-3-*methylbenzthiazole*]*methincyanine toluene*-p-*sulphonate*, from 5 : 6-methylenedioxy-2-methylbenzthiazole (3·86 g.), 2-methylthiobenzthiazole (3·62 g.), and methyl toluene-*p*-sulphonate (7·44 g.), as fine, deep-yellow needles (6·6 g.), m. p. 276° (decomp.), from methanol (1 g. in 100 c.c.) (Found: S, 16·7. $C_{25}H_{22}O_5N_2S_2$ requires S, 16·8%), [3-*ethylbenzthiazole*][5 : 6-*methylenedioxy*-3-*ethylbenzthiazole*]*methincyanine bromide*, from 5 : 6-methylenedioxy-2-methylbenzthiazole (3·86 g.), 2-methylthiobenzthiazole (3·8 g.), and ethyl toluene-*p*-sulphonate (8·3 g.), as yellow rods (4·4 g.), m. p. 301° (decomp.), from methanol (1 g. in 150 c.c.) (Found: Br, 17·4. $C_{20}H_{19}O_2N_2BrS_2$ requires Br, 17·3%); [3-*methylbenzthiazole*][5-*dimethylamino*-3-*methylbenzthiazole*]-*methincyanine bromide*, from 5-dimethylamino-2-methylbenzthiazole (Bogert and Updike, *J. Amer. Chem. Soc.*, 1927, **49**, 1373) (0·96 g.), 2-methylthiobenzthiazole (0·9 g.), and methyl toluene-*p*-sulphonate (1·86 g.), as dark-yellow rhombs (1 g.) m. p. 300° (decomp.), from methanol (1 g. in 50 c.c.) (Found: Br, 14·6. $C_{19}H_{20}N_3BrS_2$ requires Br, 14·3%), [3-*ethylbenzthiazole*][5-*dimethylamino*-3-*ethylbenzthiazole*]-

methincyanine iodide, from 5-dimethylamino-2-methylbenzthiazole (0·96 g.), 2-ethylthiobenzthiazole (0·98 g.), and ethyl toluene-*p*-sulphonate (2·0 g.), as ochre-coloured needles and rods (0·1 g.), m. p. 286° (decomp.), from methanol (60 c.c. for 1 g.) (Found: I, 24·7. $C_{21}H_{24}N_2IS_2$ requires I, 24·9%); [3-*methylbenzthiazole*][5-*diethylamino*-3-*methylbenzthiazole*]*methincyanine iodide*, from 5-diethylamino-2-methylbenzthiazole (2·2 g.), 2-methylthiobenzthiazole (1·81 g.), and methyl toluene-*p*-sulphonate (2·72 g.) yellow (2 g.), m. p. 267° (decomp.) [from methanol (1 g. in 25 c.c.)] (Found: I, 25·0. $C_{21}H_{24}N_2IS_2$ requires I, 24·9%), and [3-*ethylbenzthiazole*][5-*diethylamino*-3-*ethylbenzthiazole*]*methincyanine iodide*, from 5-diethylamino-2-methylbenzthiazole (Bogert and Updike, *loc. cit.*) (1·1 g.), 2-ethylthiobenzthiazole (0·98 g.), and ethyl toluene-*p*-sulphonate (2·0 g.), as orange rhombs (1 g.), m. p. 284° (decomp.), (from methanol (1 g. in 50 c.c.) (Found: I, 23·4. $C_{23}H_{28}N_2IS_2$ requires I, 23·6%).

[1 : 3 : 3-*Trimethylindolenine*][3-*methylbenzthiazole*]*methincyanine Iodide*.—2-Methylthiobenzthiazole (7·24 g.), finely powdered 1 : 3 : 3-trimethylindolenine methiodide (12 g.), and methyl toluene-*p*-sulphonate (7·44 g.) were fused together for 2 hours at 120°. Pyridine (100 c.c.) was added, and the mixture boiled for 45 minutes. The solution, on pouring into water (200 c.c.), afforded an orange-red *dye*, which was filtered off, washed with hot benzene, water, and ether, and recrystallised from methanol (1 g. in 20 c.c.); pale orange rhombs (5·25 g.), m. p. 262° (decomp.) (Found: I, 28·4. $C_{20}H_{21}N_2IS$ requires I, 28·4%).

Similarly were obtained [1 : 3 : 3-*trimethylindolenine*][3-*ethylbenzthiazole*]*methincyanine perchlorate*, from 2-ethylthiobenzthiazole (1·95 g.), finely powdered 1 : 3 : 3-trimethylindolenine methiodide (3·01 g.), ethyl toluene-*p*-sulphonate (2 g.), as orange-yellow rhombs (0·42 g.), m. p. 240° (decomp.), from a small quantity of methanol (Found: Cl, 8·3. $C_{21}H_{23}O_3NS$ requires Cl, 8·2%), [3-*methylbenzthiazole*][1-*methyl*-2-*pyridine*]*methincyanine iodide*, from α-picoline (3·72 g.), 2-methylthiobenzthiazole (3·62 g.), and methyl toluene-*p*-sulphonate (11·16 g.), as orange-red rhombs (1·7 g.), m. p. 296° (decomp.), from methanol (1 g. in 100 c.c.) (Found: I, 33·3. $C_{15}H_{15}N_2IS$ requires I, 33·5%), [3-*methylbenzthiazole*]-[1-*ethyl*-2-*pyridine*]*methincyanine iodide*, from α-picoline (3·72 g.), 2-ethylthiobenzthiazole (3·9 g.), and ethyl toluene-*p*-sulphonate (12 g.), as yellow-orange rhombs, m. p. 266° (decomp.) (0·3 g.), from methanol (1 g. in 100 c.c.) (Found: I, 30·6. $C_{17}H_{19}N_2IS$ requires I, 30·9%), [3-*methylbenzthiazole*][1 : 6-*dimethyl*-2-*pyridine*]*methincyanine iodide*, from 2 : 6-lutidine (2·14 g.), 2-methylthiobenzthiazole (3·62 g.), and methyl toluene-*p*-sulphonate (7·74 g.), as orange needles (1·55 g.) and rods, m. p. 243° (decomp.), from methanol (1 g. in 30 c.c.) (Found: I, 33·2. $C_{16}H_{17}N_2IS$ requires I, 32·1%), [3-*methylbenzthiazole*]-[1-*methyl*-2-*quinoline*]*methincyanine iodide*, from quinaldine (2·86 g.), 2-methyithiobenzthiazole (3·62 g.), and methyl toluene-*p*-sulphonate (7·44 g.), as scarlet needles (0·49 g.), m. p. 266° (decomp.), from methanol (1 g. in 160 c.c.) (Found: I, 29·3. Calc. for $C_{19}H_{17}N_2IS$.: I, 29·4%), and [3-*ethylbenzthiazole*]-[3-*ethyl*-2-*quinoline*]*methincyanine iodide*, from quinaldine (2·86 g.), 2-ethylthiobenzthiazole (3·9 g.), and ethyl toluene-*p*-sulphonate (8·0 g.), as crimson needles (1·4 g.), m. p. 286° (decomp.), from methanol (1 g. in 100 c.c.) (Found: I, 27·9. Calc. for $C_{21}H_{21}N_2IS$: I, 27·6%).

[3-*Methylbenzthiazole*][1-*methyl*-4-*quinoline*]*methincyanine Iodide*.—Lepidine (0·72 g.), 2-methylthiobenzthiazole (0·91 g.), and methyl toluene-*p*-sulphonate (1·86 g.) were fused together for 3½ hours at 150°. Pyridine (20 c.c.) was added, and the mixture heated under reflux for 30 minutes and poured into a warm aqueous solution of potassium iodide (5 g. in 200 c.c.). The orange *iodide* were filtered, washed with hot benzene, water, and ether, and recrystallised from methanol (1 g. in 50 c.c.); fine crimson needles (0·3 g.), m. p. 276° (decomp.) (Found: I, 29·7, 29·84. $C_{19}H_{17}N_2IS$ requires I, 29·4%).

[3-*Ethylbenzthiazole*][1-*ethyl*-4-*quinoline*]*methincyanine iodide* was prepared similarly from lepidine (0·72 g.), 2-methylthiobenzthiazole (0·91 g.), and ethyl toluene-*p*-sulphonate (2 g.). The orange crystals obtained were recrystallised from methanol (1 g. in 40 c.c.) to give orange-red needles (0·45 g.), m. p. 287° (decomp.) (Found: I, 27·1. $C_{21}H_{21}N_2IS$ requires I, 27·6%).

[3-*Ethylbenzthiazole*][1-*methyl*-4-*quinoline*]*methincyanine Iodide*.—A mixture of lepidine methiodide (2·85 g.), 2-methylthiobenzthiazole ethiodide (2·7 g.), and ethanol (40 c.c.) was boiled until solution was effected. Fused sodium acetate (0·82 g.) was added, and boiling continued for 30 minutes. The *dye* which precipitated during the reaction was filtered off, washed with hot benzene, water, and ether, and recrystallised from methanol (1 g. in 100 c.c.). It was obtained as fine, dark-red needles (3·15 g.), m. p. 270° (decomp.) (Found: I, 28·9. $C_{20}H_{19}N_2IS$ requires I, 28·5%).

[3-*Ethylbenzthiazole*][1 : 4-*dimethyl*-2-*quinoline*]*methincyanine Iodide*.—A mixture of 2 : 4-dimethylquinoline methiodide (3 g.) 2-methylthiobenzthiazole ethiodide (7·2 g.), and ethanol (80 c.c.) was heated to boiling, fused sodium acetate (1·64 g.) added, and the mixture boiled for a further 45 minutes. The red crystals which precipitated during the reaction were filtered, and recrystallised from methanol, in which the *dye* was only sparingly soluble, as red needles (3·6 g.) m. p. 291° (decomp.) (Found: I, 27·4. $C_{21}H_{21}N_2IS$ requires I, 27·6%).

[3-*Ethylbenzthiazole*][4-*methyl*-1-*ethyl*-2-*quinoline*]*methincyanine iodide*.—This *iodide*, prepared from 2 : 4-dimethylquinoline (0·79 g.), 2-ethylthiobenzthiazole (0·98 g.), and ethyl toluene-*p*-sulphonate (2·0 g.), was obtained as ochre crystals (0·32 g.), m. p. 278° (decomp.), from methanol (1 g. in 50 c.c.) (Found: I, 27·5. $C_{22}H_{23}N_2IS$ requires I, 26·8%).

[3-*Methylbenzthiazole*][6-*ethoxy*-1-*ethyl*-2-*quinoline*]*methincyanine Iodide*.—Starting materials were 6-ethoxyquinaldine (0·87 g.), 2-ethylthiobenzthiazole (0·98 g.), and ethyl toluene-*p*-sulphonate (2·0 g.). The resultant sticky mass was diluted with pyridine (20 c.c.), heated under reflux for 30 minutes, and poured into warm aqueous potassium iodide solution (5 g. in 50 c.c.). The orange precipitate was filtered off and washed with hot benzene, water, and ether. The *iodide* (0·9 g.) was obtained as orange rods, m. p. 270° (decomp.), from methanol (1 g. in 75 c.c.) (Found: I, 25·5. $C_{23}H_{25}ON_2IS$ requires I, 25·2%).

Bis-[3-*methyl*-β-*naphthothiazole*]*methincyanine Toluene*-p-*sulphonate*.—This salt was prepared from 2-methylthio-β-naphthothiazole (2·21 g.), 2-methyl-β-naphthothiazole (2·0 g.), and methyl toluene-*p*-sulphonate (3·72 g.). The *dye* was obtained by boiling out with methanol (in which it was only sparingly soluble) as minute yellow needles (2·23 g.), m. p. 293° (decomp.) (Found: S, 16·2. $C_{32}H_{28}O_3N_2S_3$ requires S, 16·5%).

Bis-[3-*ethyl*-β-*naphthothiazole*]*methincyanine Bromide*.—2-Ethylthio-β-naphthothiazole (2·35 g.),

2-methyl-β-naphthothiazole (2·0 g.), and ethyl toluene-*p*-sulphonate (4·0 g.) were used in this preparation. The *bromide* was obtained as yellow needles (1·35 g.), m. p. 263° (decomp.), from methanol (1 g. in 150 c.c.) (Found: Br, 15·5. $C_{27}H_{23}N_2BrS_2$ requires Br, 15·4%).

Bis-[3-*methyl*-α-*naphthothiazole*]*methincyanine Toluene*-p-*sulphonate*.—Similarly 2-methyl-α-naphthothiazole (1 g.), 2-methylthio-α-naphthothiazole (1·16 g.), and methyl toluene-*p*-sulphonate (2 g.) afforded a *dye*, which was obtained from methanol (1 g. in 100 c.c.) as orange-yellow needles (1·2 g.), m. p. 305° (decomp.) (Found: S, 16·6. $C_{33}H_{26}O_3N_2S_3$ requires S, 16·5%).

Bis-[3-*ethyl*-α-*naphthothiazole*]*methincyanine Bromide*.—This *salt*, prepared from 2-ethylthio-α-naphthothiazole (1·17 g.), 2-methyl-α-naphthothiazole (1·0 g.), and ethyl toluene-*p*-sulphonate (2 g.), crystallised from methanol, in which it was sparingly soluble, as yellow needles (1·4 g.), m. p. 296° (decomp.) (Found: Br, 15·5 g. $C_{33}H_{31}N_2BrS_2$ requires Be, 15·4%).

Bis-[5 : 6-*dimethoxy*-3-*methylbenzthiazole*]*methincyanine Toluene*-p-*sulphonate*.—2-Methylthio-5 : 6-dimethoxybenzthiazole (2·41 g.), 5 : 6-dimethoxy-2-methylbenzthiazole (2·09 g.), and methyl toluene-*p*-sulphonate (7·5 g.) were fused together for 3½ hours at 165—170°. Pyridine (40 c.c.) was added, and the mixture boiled for 30 minutes and poured into water; the *dye* was filtered off, washed with hot benzene, water, ethanol and ether, and crystallised from methanol (1 g. in 200 c.c.); orange rods (4·3 g.), m. p. 310° (decomp.) (Found: S, 15·9. $C_{35}H_{30}O_7N_2S_3$ requires S, 15·5%).

Similarly were prepared: *bis*-[5 : 6-*dimethoxy*-3-*ethylbenzthiazole*]*methincyanine bromide*, from 2-ethylthio-5 : 6-dimethoxybenzthiazole (2·55 g.), 5 : 6-dimethoxy-2-methylbenzthiazole (2·09 g.), and ethyl toluene-*p*-sulphonate (4·0 g.), as fine yellow needles (3·6 g.), m. p. 306° (decomp.), from methanol (1 g. in 60 c.c.) (Found: Br, 14·6. $C_{33}H_{27}O_4N_2BrS_2$ requires Br, 14·8%); [5 : 6-*dimethoxy*-3-*methylbenzthiazole*][1-*methyl*-2-*pyridine*]*methincyanine iodide*, from α-picoline (3·72 g.), 2-methylthio-5 : 6-dimethoxybenzthiazole (4·82 g.), and methyl toluene-*p*-sulphonate (11·2 g.), large orange-red needles and rods (2 g.), m. p. 291° (decomp.), from methanol (1 g. in 200 c.c.) (Found: I, 28·4. $C_{17}H_{19}O_2N_2IS$ requires I, 28·7%); [5 : 6-*dimethoxy*-3-*ethylbenzthiazole*][1-*ethyl*-2-*pyridine*]*methincyanine iodide*, from α-picoline (3·72 g.), 1-ethylthio-5 : 6-dimethoxybenzthiazole (7·65 g.), and ethyl toluene-*p*-sulphonate (12 g.), orange needles (1 g.), m. p. 243° (decomp.), from methanol (1 g. in 100 c.c.) (Found: I, 26·7. $C_{19}H_{23}O_2N_2IS$ requires I, 27·0%); and [5 : 6-*dimethoxy*-3-*methylbenzthiazole*][1-*methyl*-2-*quinoline*]-*methincyanine bromide*, from 2-methylthio-5 : 6-dimethoxybenzthiazole (1·21 g.), quinaldine (0·72 g.), and methyl toluene-*p*-sulphonate (1·86 g.), orange (1·7 g.), m. p. 242° (decomp.) [from methanol (1 g. in 60 c.c.)] (Found: Br, 18·0. $C_{21}H_{21}O_2N_2BrS$ requires Br, 18·0%).

[5 : 6-*Dimethoxy*-2-*ethylbenzthiazole*][1-*ethyl*-2-*quinoline*]*methincyanine Toluene*-p-*sulphonate*.—Quinaldine (4·29 g.), 2-methylthio-5 : 6-dimethoxybenzthiazole (7·23 g.), and ethyl toluene-*p*-sulphonate (12 g.) were fused together for 4 hours at 140°. The red solid obtained was boiled with ethanol (180 c.c.), anhydrous sodium acetate (3·28 g.) added, and the mixture heated under reflux for 15 minutes. The mixture was poured into water, and the precipitate filtered off, washed with hot benzene, ethanol, and ether, and recrystallised from methanol (1 g. in 150 c.c.) to give fine crimson needles (4·1 g.), m. p. 296° (decomp.) (Found: S, 11·6. $C_{30}H_{32}O_6N_2S_2$ requires S, 11·4%).

Similar preparations afforded: *bis*-[5 : 6-*methylenedioxy*-3-*methylbenzthiazole*]*methincyanine toluene*-p-*sulphonate*, from 2-methylthio-5 : 6-methylenedioxybenzthiazole (2·25 g.), 5 : 6-methylenedioxy-2-methylbenzthiazole (1·93 g.), and methyl toluene-*p*-sulphonate (3·75 g.), fine yellow needles (3·4 g.), m. p. 316° (decomp.), from methanol (1 g. in 200 c.c.) (Found: S, 16·7. $C_{35}H_{32}O_7N_2S_3$ requires S, 16·9%); *bis*-[5 : 6-*methylenedioxy*-3-*ethylbenzthiazole*]*methincyanine bromide*, from 2-ethylthio-5 : 6-methylenedioxybenzthiazole (2·39 g.), 5 : 6-methylenedioxy-2-methylbenzthiazole (1·93 g.), and ethyl toluene-*p*-sulphonate (4·05 g.), fine, ochre-coloured needles (1·3 g.), m. p. 360° (decomp.), from methanol (1 g. in 200 c.c.) (Found: S, 15·9. $C_{35}H_{32}O_8N_2S_2$ requires S, 16·0%); [5 : 6-*methylenedioxy*-3-*methylbenzthiazole*][1-*methyl*-2-*pyridine*]*methincyanine iodide*, from α-picoline (3·72 g.), 2-methylthio-5 : 6-methylenedioxybenzthiazole (4·5 g.), and methyl toluene-*p*-sulphonate (11·2 g.), sparingly soluble, brown rhombs (1·1 g.), m. p. 305° (decomp.), from methanol (1 g. in 200 c.c.) (Found: I, 30·0. $C_{18}H_{15}O_4N_2SI$ requires I, 29·8%); and [5 : 6-*methylenedioxy*-3-*ethylbenzthiazole*][1-*ethyl*-2-*pyridine*]-*methincyanine iodide*, from α-picoline (3·72 g.), 2-ethylthio-5 : 6-methylenedioxybenzthiazole (4·78 g.), and ethyl toluene-*p*-sulphonate (12 g.), orange needles (1·6 g.), m. p. 304° (decomp.), from methanol (Found: I, 28·2. $C_{19}H_{19}O_4N_2IS$ requires I, 28·0%).

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320. *The Reaction of Bromine with Silver (+)-α-Phenylpropionate. An Electrophilic Bimolecular Substitution.*

By C. L. Arcus, A. Campbell, and J. Kenyon.

The reaction of bromine in carbon tetrachloride solution with silver (+)-α-phenylpropionate yields (+)-α-phenylethyl bromide with inversion of configuration. It is considered that the positive-halogen compound, CHPhMe·CO·OBr, is first formed and that reaction subsequently proceeds according to equation (i) (below), with the similar reactions (ii) and (iii) as possible subsidiary reactions. All are bimolecular substitutions with inversion of configuration effected by the electrophilic reagent, positive bromine. The partial racemisation which occurs during this reaction is attributed to the experimentally-observed racemisation of (+)-α-phenylethyl bromide in solution in carbon tetrachloride by bromine in the presence of silver bromide.

Hell–Volhard bromination of (+)-α-phenylpropionic acid yields (±)-α-bromo-α-phenylpropionic acid.

Interaction of one equivalent of the silver salt of an aliphatic or substituted aliphatic carboxylic acid with two equivalents of bromine yields the alkyl bromide containing one carbon atom less than the original acid; numerous examples from the literature are cited by Kleinberg (*Chem. Reviews*, 1947, **40**, 386). The mechanism which has been generally accepted for this reaction (Birckenbach, Goubeau, and Berninger, *Ber.*, 1932, **65**, 1339; Bockemüller and Hoffmann, *Annalen*, 1935, **519**, 165; Kleinberg, *loc. cit.*) is that an intermediate positive-halogen compound R·CO·OBr, the acyl hypobromite, is formed:

$$R{\cdot}CO_2Ag + Br_2 \longrightarrow R{\cdot}C(=O){-}OBr + AgBr$$

This undergoes decarboxylation to give the alkyl bromide, RBr.

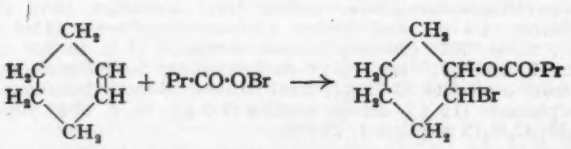
Bockemüller and Hoffman (*loc. cit.*) obtained the following evidence for the existence of the acyl hypobromites. Silver butyrate was shaken with bromine in carbon tetrachloride solution, and the product filtered. The yellowish-green filtrate, which was free from silver, liberated iodine from acidified potassium iodide solution, whereby its oxidising power was determined. The total bromine content was one-half of that of a solution of bromine of equal oxidising power,

$$Pr{\cdot}CO{\cdot}OBr + 2HI \longrightarrow Pr{\cdot}CO_2H + HBr + I_2$$

as compared with

$$Br_2 + 2HI \longrightarrow 2HBr + I_2.$$

Further, the butyryl hypobromite so prepared combined with *cyclo*hexene to give 2-bromo-*cyclo*hexyl butyrate:



The use of a silver-free filtrate (as distinct from a suspension of the silver salt in bromine–carbon tetrachloride) precluded the possibility that the 2-bromo*cyclo*hexyl butyrate might be formed as follows:

$$-CH{:}CH- + Br_2 \longrightarrow -CHBr{\cdot}C^{\oplus}H- + Br^{\ominus}$$

$$-CHBr{\cdot}C^{\oplus}H- + Pr{\cdot}CO_2^{\ominus}Ag^{\oplus} \longrightarrow -CHBr{\cdot}CH(O{\cdot}CO{\cdot}Pr)- + AgBr.$$

Birckenbach *et al.* (*loc. cit.*) had earlier effected the reaction of silver acetate and iodine in ether at −80° and by adding *cyclo*hexene to the filtrate had obtained 2-iodo*cyclo*hexyl acetate.

Determinations of oxidising power and of bromine content, and addition to *cyclo*hexene, gave analogous results with a solution of benzoyl hypobromite, which was similarly prepared.

These findings demonstrate the existence in solution of compounds of the structure R·CO·OBr and indicate the bromine to be a " positive " halogen such as exists in hypobromous acid and the *N*-bromoamides and in contrast with the bromine of alkyl and acyl bromides.

In order to elucidate further the mechanism of the substitution of carboxyl by bromine, silver α-phenylpropionate, prepared from the acid having $\alpha^{15°}_{5893}$ +42·3° (*l*, 0·5) (optical purity, 81·7%), was treated in boiling carbon tetrachloride suspension with two equivalents of bromine;

it gave, in good yield, α-phenylethyl bromide, $\alpha_{5893}^{16°}$ +46·2° (*l*, 1·0) [optical purity, 35·2%, by comparison with the highest recorded rotatory power of this compound, $\alpha_{5893}^{18°}$ +131·4° (*l*, 1·0); Gerrard, *J.*, 1946, 741].

The retention of asymmetry during this substitution is thus 43·1%, and the following data permit comparison of the configurations of the acid and the bromide. (+)-α-Phenylpropionic acid has been converted, with retention of configuration, into (−)-α-phenylethylamine by the Hofmann, and other, rearrangements (Arcus and Kenyon, *J.*, 1939, 916; Kenyon and Young, *J.*, 1941, 263; Campbell and Kenyon, *J.*, 1946, 25). With nitrous acid the (−)-amine has been found (Campbell, unpublished results) to yield (+)-α-phenylethyl alcohol with 14·8% retention of asymmetry; it is concluded that during this reaction the diazonium ion $CHMePh{\cdot}N_2^{\oplus}$ dissociated into nitrogen and the ion $^{\oplus}CHPhMe$, and that the latter reacts with water to form the alcohol with a preponderance of the inverted configuration, due to shielding of the original point of attachment by the released nitrogen molecule. Finally, (+)-α-phenylethyl alcohol and (+)-α-phenylethyl bromide have the same configuration (Hughes, Ingold, and Scott, *J.*, 1937, 1201). Thus (+)-α-phenylpropionic acid and (+)-α-phenylethyl bromide have opposite configurations, and the silver salt–bromine reaction proceeds with inversion of configuration.

The following mechanism for the reaction is put forward. α-Phenylpropionyl hypobromite, CHPhMe·CO·OBr, is first formed, and two molecules of this compound react according to equation (i) to yield α-phenylethyl bromide, carbon dioxide, and two ions which combine to regenerate one molecule of α-phenylpropionyl hypobromite:

(i) $CHPhMe{\cdot}CO{\cdot}O{-}Br + Me{-}\underset{Ph}{\overset{H}{C}}{-}C(=O){-}O{-}Br \longrightarrow CHPhMe{\cdot}CO_2^{\ominus} + Br{-}\underset{Ph}{\overset{H}{C}}{-}Me + CO_2 + Br^{\oplus}$

followed by

$$CHPhMe{\cdot}CO_2^{\ominus} + Br^{\oplus} \longrightarrow CHPhMe{\cdot}CO{\cdot}OBr$$

(ii) $Br{-}Br + CHPhMe{-}CO{\cdot}O{-}Br \longrightarrow Br^{\ominus} + Br{-}CHPhMe + CO_2 + Br^{\oplus}$

followed by

$$Br^{\ominus} + Br^{\oplus} \longrightarrow Br_2$$

(iii) $Br^{\oplus} + CHPhMe{-}CO{\cdot}O{-}Br \longrightarrow Br{-}CHPhMe + CO_2 + Br^{\oplus}$

It is possible that molecular bromine may react by a similar mechanism (ii) to yield α-phenylethyl bromide, carbon dioxide, and ions which combine to regenerate bromine. Finally, a small concentration of bromine cations may be either set up by reactions (i) and (ii) or may exist in equilibrium with CHPhMe·CO·OBr or with Br_2, and reaction may then proceed by (iii) in which α-phenylethyl bromide and carbon dioxide are formed and bromine cation is regenerated. All the reactions (i), (ii), and (iii) yield α-phenylethyl bromide with inversion of configuration and in each of them the attacking reagent is positive bromine, *i.e.*, bromine which tends to separate or which has separated with a sextet of electrons.

The configuration of the α-phenylethyl bromide resulting from these reactions would be completely inverted; the bromide obtained experimentally was inverted, but also partly racemised. The possibility that there may be a subsidiary mechanism of reaction in which a planar α-phenylethyl fragment is formed at one stage or which proceeds with retention of configuration is not excluded, but it appears most probable that the racemisation is due to the action of bromine and silver bromide on the fully inverted (+)-α-phenylethyl bromide during the period elapsing between its formation and the completion of the experiment. Experimental results leading to this explanation are described below.

It is concluded that the silver α-phenylpropionate–bromine reaction is mainly or entirely a bimolecular substitution with inversion of configuration effected by the electrophilic reagent, positive bromine. It appears to be the first established example of this class of replacement reaction.

The following experiments were carried out in order to elucidate the cause of the partial racemisation which occurred during the silver salt reaction. (+)-α-Phenylethyl bromide was dissolved in carbon tetrachloride and heated under reflux for 1 hour with silver bromide and bromine (0·7 equivalent); the recovered α-phenylethyl bromide was 99·8% racemised. During this experiment some hydrogen bromide was evolved, indicating the occurrence of bromination. From a similar experiment, in which silver bromide but no bromine was used, there was obtained, after heating for 3 hours, α-phenylethyl bromide which was racemised only to the extent of 0·6%. A similar experiment in which bromine (2·0 equivalents) but no silver bromide

was used gave, after 3 and 19 hours, respectively, 38·4 and 99·0% racemisation. Thus racemisation is rapid when bromine and silver bromide are present together, slower with bromine alone, and slight with silver bromide alone.

The mechanism of racemisation of α-phenylethyl bromide by bromine, with catalysis by silver bromide, is uncertain. It may be due to bimolecular halogen exchange, which has been shown (Hughes, Juliusberger, Scott, Topley, and Weiss, *J.*, 1936, 1173) to be the mechanism of the racemisation of α-phenylethyl bromide by lithium bromide in solution in acetone. Alternatively, it may be due to a free radical reaction, $RBr + Br_2 \longrightarrow R^{\bullet} + Br_2 + Br^{\bullet}$, followed by $R^{\bullet} + Br^{\bullet} \longrightarrow RBr$.

Further work bearing on the mechanism of this racemisation is in progress.

A Hell–Volhard bromination of (+)-α-phenylpropionic acid has been carried out by converting the acid into the acid bromide and heating the latter with bromine; the resultant bromo-acid bromide with water gave optically inactive α-bromo-α-phenylpropionic acid. The isolation of the acid involved a crystallisation, so that the optically active acid, had it been present in small proportion, might have been removed; further, the reaction conditions might have caused racemisation of the optically active acid after its formation. With these reservations, the formation of optically-inactive α-bromo-α-phenylpropionic acid is consistent with bromination *via* the (symmetrical) enolic form of α-phenylpropionyl bromide, a mechanism of reaction which has been discussed by Watson (" Modern Theories of Organic Chemistry," 2nd edition, Oxford, 1941, p. 166).

Experimental.

(±)-α-Phenylpropionic acid was prepared by the method of Campbell and Kenyon (*loc. cit.*) and resolved according to Arcus and Kenyon (*loc. cit.*).

Silver-salt–Bromine Reaction.—α-Phenylpropionic acid having $\alpha_{5893}^{15°}$ +42·3° (*l*, 0·5) (11·2 g.) was dissolved in one equivalent of N-NaOH (75 ml.), and to this solution was added silver nitrate solution until no further precipitate resulted. The grey precipitate was filtered off, washed, and dried *in vacuo* (yield, 18·0 g.). This silver salt was suspended with stirring in boiling, dry carbon tetrachloride (100 ml.), to which was added dry bromine (11·1 g., 2·0 equivs.). The solution was heated under reflux until all the colour had disappeared and then for a further hour. It was filtered from silver bromide, washed with sodium hydrogen carbonate solution and then with water, and dried (Na_2SO_4). Fractionation gave (+)-α-phenylethyl bromide, b. p. 98—99°/20 mm. (7·1 g.), having $\alpha_{5893}^{16°}$ +46·2° (*l*, 1·0).

Racemisation of (+)-α-Phenylethyl Bromide.—A specimen of α-phenylethyl bromide, kindly supplied by Dr. W. Gerrard, had b. p. 80·5—83·5°/8 mm. and $\alpha_{5893}^{16°}$ +96·6° (*l*, 1·0). This (3·3 g.) was dissolved in dry carbon tetrachloride (48 ml.), the solution having $\alpha_{5893}^{13°}$ +8·89° (*l*, 2·0). Silver bromide (7·4 g.) and bromine (0·95 g., 0·7 equiv.) were added, and the mixture was boiled under reflux for 1 hour; acid fumes were evolved. The solution was washed with, successively, water, sodium thiosulphate solution, and water, and dried (Na_2SO_4); it had $\alpha_{5893}^{15°}$ +0·03° (*l*, 2·0). Fractionation gave α-phenylethyl bromide (1·1 g.), b. p. 86°/7 mm., $\alpha_{5893}^{15°}$ +0·21° (*l*, 1·0), and a fraction, b. p. 126°/8 mm., which solidified. The solid was dissolved in carbon tetrachloride, and light petroleum added; crystals (0·3 g.), m. p. 73—74°, separated, unchanged by further crystallisation from light petroleum. α-Phenylethyl bromide having $\alpha_{5893}^{15°}$ +104·7° (*l*, 1·0) was prepared by the method of Gerrard (*loc. cit.*). It (7 g.) was dissolved in dry carbon tetrachloride (40 ml.) and heated under reflux with powdered silver bromide (5 g.) for three hours. Fractionation gave α-phenylethyl bromide (5·4 g.), b. p. 96°/18 mm., $\alpha_{5893}^{18°}$ +104·1°. α-Phenylethyl bromide (6·5 g.) having $\alpha_{5893}^{18°}$ +104·2° (*l*, 1·0) was dissolved in carbon tetrachloride (37 ml.), bromine (5·7 g., 2·0 equivs.) was added, and the solution boiled under reflux for three hours. A portion (13 ml.) was then removed, washed with aqueous sodium thiosulphate, dried (Na_2SO_4), and fractionated; it yielded α-phenylethyl bromide (2·0 g.), b. p. 96—97°/18 mm., $\alpha_{5893}^{17°}$ +32·1° (*l*, 0·5). The remaining solution was boiled under reflux for a further sixteen hours, giving, after purification as before, α-phenylethyl bromide (3·1 g.), b. p. 96—97°/20 mm., $\alpha_{5893}^{18°}$ +0·5° (*l*, 0·5).

Hell–Volhard Bromination of (+)-α-Phenylpropionic Acid.—α-Phenylpropionic acid having $\alpha_{5893}^{15°}$ +42·3° (*l*, 0·5) (7·5 g.) was warmed to 60° for ½ hour with phosphorus tribromide (13·5 g.). The upper mobile layer was decanted and heated with bromine (8 g.) under reflux in an oil-bath at 140—150° for four hours. The resultant liquid was poured into water and then set aside overnight. An oil was produced which dissolved completely in sodium hydroxide solution. This solution was acidified and extracted with ether, and the extract was dried (Na_2SO_4) and distilled, to yield a fraction, b. p. 175—180°/25 mm. (5 g.), which partly solidified. Crystallisation from carbon disulphide by addition of light petroleum gave optically inactive α-bromo-α-phenylpropionic acid (4 g.), m. p. 91—93° (Fittig and Kast, *Annalen*, 1880, **206**, 28, record m. p. 93°).

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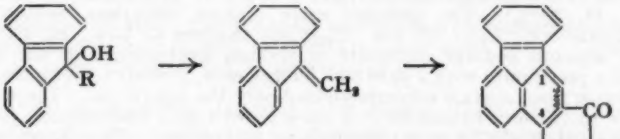
Battersea Polytechnic, London, S.W.11. [*Received, January 5th,* 1949.]

321. *Syntheses in the Fluoranthene Series.*

By Neil Campbell and H. Wang.

9-Hydroxy-9-alkylfluorenes in suitable solvents condense with dienophiles to give Diels–Alder adducts, and a number of fluoranthene derivatives have been thus synthesised. The substance considered by von Braun and Manz (*Annalen*, 1932, **496**, 170) to be naphtho-(2′ : 3′-3 : 4)fluoranthene-1′ : 4′-quinone has been shown to have some other structure and is probably 4 : 5-phthaloylfluoranthene.

When 9-hydroxy-9-methylfluorene (I; R = Me) is treated with acetic anhydride and an excess of maleic anhydride, *fluoranthene-3 : 4-dicarboxylic anhydride* (III) is produced, presumably by Diels–Alder addition to the intermediate 9-methylenefluorene (II). Spontaneous

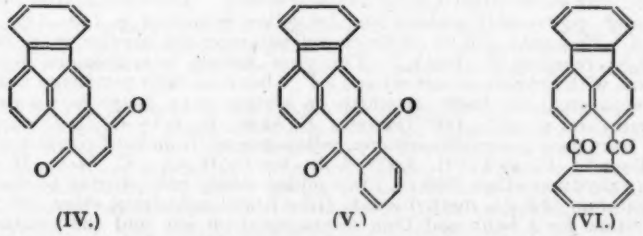


(I.) (II.) (III.)

dehydrogenation obviously occurs, and no hydrogenated intermediate was detected. The anhydride, when decarboxylated with calcium hydroxide, yielded fluoranthene, and with copper and quinoline gave an impure monocarboxylic acid which must be fluoranthene-3-carboxylic acid since the 4-carboxyl group is readily removed under the conditions used (Campbell and Easton, this vol., p. 340).

The formation of fluoranthene-3 : 4-dicarboxylic anhydride is, so far as we know, the first example of an aromatic bond in fluorene participating in the Diels–Alder reaction (cf. Wagner-Jauregg, *Ber.*, 1930, **63**, 3213; *Annalen*, 1931, **491**, 1).

9-Hydroxy-9-ethylfluorene (I; R = Et) and excess of maleic anhydride in acetic anhydride yielded a non-crystalline product, m. p. 281—285°, probably a polymer, but in nitrobenzene *2-methylfluoranthene-3 : 4-dicarboxylic anhydride* was obtained, condensation again being accompanied by dehydrogenation. Dehydrogenation in nitrobenzene is not unknown (*e.g.*, Clar, *Ber.*, 1932, **65**, 846; 1936, **69**, 1686), but the dehydrogenation in acetic anhydride was less easily anticipated. Decarboxylation of the anhydride gave *2-methylfluoranthene*. No difficulty was encountered in preparing *9-hydroxy-9-methyl-2-ethylfluorene*, the ether solution of which on evaporation on the water-bath yielded *2-ethyl-9-methylenefluorene*. In contrast with 9-methylenefluorene (cf. Wieland *et al.*, *Ber.*, 1922, **55**, 3313; *Annalen*, 1937, **530**, 274), this compound is quite stable. Attempts to condense the hydroxy-compound with maleic anhydride in acetic anhydride or nitrobenzene were unsuccessful, only polymeric products being obtained.



(IV.) (V.) (VI.)

With *p*-benzoquinone and α-naphthaquinone as dienophiles 9-hydroxy-9-methylfluorene gave *3 : 4-benzfluoranthene-1′ : 4′-quinone* (IV) and *naphtho(2′ : 3′-3 : 4)fluoranthene-1′ : 4′-quinone* (V), respectively. The constitution of the latter quinone (orange-red, m. p. 249—251°) follows unequivocally from its synthesis, and consequently the yellow quinone, m. p. 328—331°, obtained by von Braun and Manz (*loc. cit.*) by the cyclisation of 4-*o*-carboxybenzoylfluoranthene must have some constitution other than that of (V) assigned by these authors. It is suggested that their ring-closure occurred not at the 3-, but at the 5-, position to form 4 : 5-phthaloyl-fluoranthene (VI), and that the bluish-violet vat obtained by von Braun and Manz with sodium dithionite was due to an impurity. The formation of similar phthaloyl compounds has been noted in the naphthalene series (Rieche *et al.*, *Ber.*, 1932, **65**, 1371; B.P. 303,375), and

preliminary ring-closure experiments with 3-*o*-carboxybenzoylacenaphthene indicate that one of the products is 3 : 4-phthaloylacenaphthene. Graebe and Perutz (*Annalen*, 1903, **327**, 99) effected ring-closure of 3-*o*-carboxybenzoylacenaphthene with phosphorus pentachloride and, without proof, assigned to the product the structure naphtho(2′ : 3′-2 : 3)acenaphthene-1′ : 4′-quinone. The matter is being further investigated.

Experimental.

All analyses were done by Drs. Weiler and Strauss, Oxford. Fluorescence observations were made under a Hanovia ultra-violet lamp.

Fluoranthene-3 : 4-*dicarboxylic Anhydride*.—9-Hydroxy-9-methylfluorene (12·0 g.), maleic anhydride (30 g.), and acetic anhydride (80 c.c.) were heated under reflux for 2 hours. *Fluoranthene*-3 : 4-*dicarboxylic anhydride* separated on cooling and crystallised from acetic anhydride in yellow needles, m. p. 267—268°, with an orange fluorescence (yield, 1·60 g.; 10%) (Found: C, 79·2; H, 3·3. $C_{18}H_8O_3$ requires C, 79·4; H, 3·0%). The *dimethyl* ester formed colourless needles (from methanol), m. p. 118—119° (Found: C, 75·0; H, 4·3. $C_{20}H_{14}O_4$ requires C, 75·4; H, 4·4%). The anhydride dissolved in warm aqueous sodium carbonate or sodium hydroxide, and the alkaline solution on acidification yielded a precipitate with a pale blue fluorescence, probably fluoranthene-3 : 4-dicarboxylic acid, but crystallisation from organic solvents yielded only the anhydride. The anhydride (0·5 g.) was heated to red heat with calcium hydroxide (5 g.) in a Pyrex tube; fluoranthene (50% yield) sublimed up the tube and was identified by its m. p., mixed m. p., and picrate. The anhydride (0·4 g.) was heated in an oil-bath at 180° with quinoline (20 c.c.) and copper bronze (0·1 g.) for ¼ hour; the mixture was filtered and treated with 10% sodium hydroxide (10 c.c.), and the quinoline removed by steam-distillation. The solution was acidified, and the gelatinous precipitate crystallised from benzene and light petroleum (b. p. 60—80°), to give impure fluoranthene-3-carboxylic acid, m. p. 240—243°, subliming in yellow needles with a pale blue fluorescence (Found: C, 81·1; H, 3·8. Calc. for $C_{17}H_{10}O_2$: C, 82·9; H, 4·1%).

Synthesis of 2-*Methylfluoranthene*.—9-Hydroxy-9-ethylfluorene (0·5 g.), nitrobenzene (20 c.c.), and maleic anhydride (1·5 g.) were boiled under reflux for 2½ hours, the solution on cooling deposited 2-*methylfluoranthene*-3 : 4-*dicarboxylic anhydride*, which was washed with ethanol and crystallised from tetralin in long yellow needles, m. p. 275—280° (decomp.), with a yellow fluorescence (yield, 0·28 g.; 41%) (Found: C, 78·65; H, 3·6. $C_{19}H_{10}O_3$ requires C, 79·7; H, 3·5%). The anhydride (0·2 g.) was heated with calcium hydroxide; 2-*methylfluoranthene* sublimed along the tube and after purification on a short column of alumina crystallised from light petroleum (b. p. 60—80°) in pale yellow needles, m. p. 72—73°, with a vivid blue fluorescence (yield, 0·10 g.; 66%) (Found: C, 94·4; H, 5·75. $C_{17}H_{12}$ requires C, 94·4; H, 5·6%). The *picrate* formed orange needles, m. p. 207—208° (Found: N, 9·35. $C_{23}H_{15}O_7N_3$ requires N, 9·4%).

Attempted Synthesis of 4-*Ethylfluoranthene*.—2-Acetylfluorene (25 g.), toluene (100 c.c.), water (35 c.c.), and concentrated hydrochloric acid (50 c.c.) were added to zinc dust (60 g.) which had been amalgamated with 5 g. of mercuric chloride in water (75 c.c.) and concentrated hydrochloric acid (2½ c.c.); the mixture was heated under reflux for 24 hours with the addition of concentrated hydrochloric acid (25 c.c.) every 6 hours. The cold solution was extracted with ether, and the dried ethereal extract evaporated. A brown semi-solid oil was obtained and gave a fraction, b. p. 170—175°/15 mm., which solidified and, when crystallised from light petroleum (b. p. 40—60°), gave colourless plates of 2-*ethylfluorene*, m. p. 81—82° (yield, 10·4 g.; 45%). This sublimed readily and had a bright blue fluorescence in solution (Found: C, 92·8; H, 6·9. $C_{15}H_{14}$ requires C, 92·7; H, 7·3%). Sodium dichromate (12·5 g.) in acetic acid (25 c.c.) was slowly added to a boiling solution of 2-ethylfluorene (12·0 g.) in acetic acid (25 c.c.), and the solution heated under reflux for 2 hours. The cold solution was extracted with ether, and the ethereal layer washed with water, dried ($CaCl_2$), and evaporated. The residue, after three crystallisations from light petroleum (b. p. 60—80°), yielded 5·5 g. of yellow prisms, m. p. 115—119°, consisting mainly of 2-ethylfluorenone. This gave a 2 : 4-*dinitrophenylhydrazone*, red needles, m. p. 267—268° (Found: N, 13·8. $C_{21}H_{16}O_4N_4$ requires N, 14·4%). The pure ketone was obtained by chromatographic separation on alumina with benzene as solvent and a 1 : 3 benzene–light petroleum mixture as developer. Two yellow zones separated, the lower of which on elution gave 2-*ethylfluorenone* as yellow plates (from light petroleum), m. p. 127—128° (Found: C, 85·9; H, 5·9. $C_{15}H_{12}O$ requires C, 86·5; H, 5·8%). The upper band gave 2-acetylfluorenone, yellow prisms (from light petroleum), m. p. 156—157° (lit., 154—155°) (Found: C, 80·4; H, 4·95. Calc. for $C_{15}H_{10}O_2$: C, 81·1; H, 4·5%). 2-Ethylfluorenone (6 g.) in anhydrous ether (300 c.c.) was added slowly with stirring to the Grignard reagent prepared from magnesium (2·0 g.), methyl iodide (16 g.), and anhydrous ether (50 c.c.); the solution was heated under reflux for ½ hour and then decomposed on ice, and the solution acidified. The ethereal layer was washed with aqueous sodium hydrogen sulphite and then with water, and dried. On evaporation at room temperature it yielded 9-*hydroxy*-9-*methyl*-2-*ethylfluorene*, which crystallised from benzene in needles, m. p. 124—125° (yield, 2·8 g.; 43%) (Found: C, 85·7; H, 6·8. $C_{16}H_{16}O$ requires C, 85·7; H, 7·2%). Evaporation of the solution on the water-bath, however, gave 2-*ethyl*-9-*methylenefluorene*, plates (from benzene), m. p. 156—157° (Found: C, 93·0; H, 6·75; *M*, 220. $C_{16}H_{14}$ requires C, 93·2; H, 6·8; *M*, 206).

3 : 4-*Benzfluoranthene*-1′ : 4′-*quinone*.—9-Hydroxy-9-methylfluorene (2·5 g.), *p*-benzoquinone (10 g.), and acetic anhydride (50 c.c.) were heated under reflux for 2 hours. When the solution cooled, orange-red needles of the *quinone* separated and were crystallised from glacial acetic acid; m. p. 245—246°; yield, 0·90 g. (25%) (Found: C, 84·1; H, 3·65. $C_{22}H_{10}O_2$ requires C, 85·1; H, 3·6%). The quinone gives a purple colour in concentrated sulphuric acid and a wine-red vat with sodium dithionite.

Naphtho(2′ : 3′-3 : 4)*fluoranthene*-1′ : 4′-*quinone*.—9-Hydroxy-9-methylfluorene (2·5 g.), α-naphthaquinone (10 g.), and acetic anhydride (50 c.c.) were heated under reflux for 2 hours; on cooling, the

mixture deposited orange-red needles of the *quinone* which was crystallised from nitrobenzene and light petroleum (b. p. 100—120°); m. p. 249—251°; yield 0·50 g. (12%) (Found: C, 86·35; H, 3·7. $C_{34}H_{12}O_2$ requires C, 86·7; H, 3·6%). The quinone gives a bluish-green colour with concentrated sulphuric acid and a bluish-violet vat with sodium dithionite.

Thanks are due to the Department of Scientific and Industrial Research for a maintenance grant to one of us (H. W.) and to the Anglo-Iranian Oil Co., Ltd., for a grant.

UNIVERSITY OF EDINBURGH. [*Received, January 5th*, 1949.]

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322. *The Reactions of Substituted Maleimides with Thiols.*

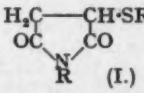
By D. H. MARRIAN.

The condensation of *N*-ethyl- and *N*-phenyl-maleimide with mercaptoacetic acid and with *o*-mercaptobenzoic acid gives products of structure (I) in good yield. Desulphurisation by Raney nickel has given the expected degradation products.

IT has been reported (Friedmann, Marrian, and Simon-Reuss, *Brit. J. Pharmacol.*, 1949, **4**, 105) that the mixing of equimolar neutral solutions of certain maleimides and of mercaptoacetic acid or glutathione at room temperature leads to the rapid removal of free thiol groups from solution.

Whereas it has been shown by Morgan and Friedmann (*Biochem. J.*, 1938, **32**, 733) that under similar conditions the reaction of such thiols with maleic acid is slow and incomplete (some 50% after 5 hours at 37°), the reaction of substituted maleimides with thiols is substantially complete in ½—2 minutes. In view of the interesting antimitotic activity shown by certain imides on cultures of chick fibroblasts (Friedmann, Marrian, and Simon-Reuss, *loc. cit.*) it was considered of interest to isolate and characterise some of the condensation products, especially since the thiol adducts of maleic acid still show some of the antimitotic activity of the parent unsubstituted maleic acid (*idem, Brit. J. Pharmacol.*, 1948, **3**, 335).

The imides used in this investigation were *N*-ethyl- and *N*-phenyl-maleimide (being more readily available than the parent compound), and the condensations with mercaptoacetic acid and with *o*-mercaptobenzoic acid were investigated in each case. Addition products of structure



(I)

(*a*) R = Et; R′ = $CH_2 \cdot CO_2H$.
(*b*) R = Et; R′ = $C_6H_4 \cdot CO_2H$.
(*c*) R = Ph; R′ = $CH_2 \cdot CO_2H$.
(*d*) R = Ph; R′ = $C_6H_4 \cdot CO_2H$.

(I) were isolated in good yield. Raney nickel in boiling alcohol caused fission of the S–C bonds, giving a substituted succinimide and acetic or benzoic acid, although often in low yield. From (I*a*) and (I*b*) the low-melting *N*-ethylsuccinimide was identified by alkaline hydrolysis to succinic acid.

EXPERIMENTAL.

N-Ethylmaleimide.—An adaptation of the method of Piutti (*Gazzetta*, 1888, **18**, 483) proved reliable. *N*-Ethylmaleamic acid (10 g.) and medicinal paraffin (20 c.c.) were heated to 200° in a metal-bath. The lachrymatory distillate crystallised and was pressed on a porous plate and redistilled, to give 3·8 g. of the imide, m. p. 44°.

N-Phenylmaleimide was prepared by an adaptation of the method of Auwers (*Annalen*, 1899, **309**, 346). An intimate mixture of *N*-phenylmaleamic acid (5 g.) (Anschutz and Wurtz, *Annalen*, 1887, **239**, 140) and phosphoric oxide (5 g.) was packed into a 25-c.c. distilling flask with glass wool, attached to an air-condenser and receiver, and evacuated at the water pump. The flask was immersed in a metal-bath at 100° and heated fairly rapidly to 250°; during this operation a yellow oil distilled, which quickly solidified (2·2 g.). Larger batches gave much lower yields. The crude distillate crystallised from 50% aqueous alcohol (18 c.c.) in long thin prisms (1·45 g.), m. p. 86—88°.

N-Ethyl-α-carboxymethylthiosuccinimide.—*N*-Ethylmaleimide (1·25 g.) in water (100 c.c.) was treated with a solution of mercaptoacetic acid (0·92 g., 1 mol.), neutralised to phenolphthalein with N-sodium hydroxide (*ca.* 8 c.c.), in water (50 c.c.). After 30 minutes at room temperature, the clear solution was evaporated *in vacuo* at 60—70°, and the residual glass dissolved in water (15 c.c.), acidified to Congo-red with 5N-sulphuric acid, and extracted continuously with ether. The extract was dried (Na_2SO_4) and evaporated, finally *in vacuo* at 60°. The oily residue crystallised on treatment with a little alcohol (yield, 2·24 g., 100%). Recrystallisation from dry benzene gave colourless prisms (2·0 g.), m. p. 88—89°. For analysis, the *compound* was recrystallised from dry benzene to m. p. 90—91° (Found, in material dried at room temperature: C, 44·1, 44·3; H, 5·0, 4·6; N, 6·8, 6·9. $C_8H_{11}O_4NS$ requires C, 44·2; H, 5·1; N, 6·4%).

N-Ethyl-α-o-carboxyphenylthiosuccinimide.—*N*-Ethylmaleimide (125 mg.) and *o*-mercaptobenzoic acid (154 mg., 1 mol.) in alcohol (4 c.c.) were set aside overnight at room temperature. On gentle scratching, crystallisation ensued. The *product*, after filtration and washing with alcohol, formed

microcrystalline prisms (150 mg.; 54%), which recrystallised from methanol in almost colourless prisms, m. p. 161·5—163·5° (Found, in material dried at 80°: C, 55·7; H, 4·8; N, 5·4. $C_{13}H_{13}O_4NS$ requires C, 55·9; H, 4·7; N, 5·0%).

N-Phenyl-α-carboxymethylthiosuccinimide.—*N*-Phenylmaleimide (176 mg.), suspended in water (5 c.c.), was treated with a solution of mercaptoacetic acid (92 mg., 1 mol.) in water (1 c.c.) and N-sodium hydroxide (1 c.c.). The imide quickly dissolved when the mixture was shaken, to give a colourless solution. After 15 minutes, a trace of insoluble material was filtered off, and the filtrate acidified with 5N-sulphuric acid. A colourless crystalline solid was precipitated, which was filtered off and washed with a little water. The *product* crystallised from water (3 c.c.) in colourless microcrystalline prisms, m. p. 153·5—154·5°. For analysis, the compound was again recrystallised from water; m. p. unchanged (Found, in material dried at 80°: C, 54·1; H, 4·1; N, 5·7; equiv., 259. $C_{12}H_{11}O_4NS$ requires C, 54·3; H, 4·2; N, 5·3%; equiv., 265·3).

N-Phenyl-α-o-carboxyphenylthiosuccinimide.—This *imide*, prepared as was its *N*-ethyl analogue (above) formed colourless prisms, m. p. 179·5—182° [300 mg. (90%) from 176 mg. of phenylmaleimide]. Recrystallisation from alcohol (4 c.c.) gave colourless prisms, m. p. 179—180° (Found, in material dried at 80°: C, 62·4; H, 4·1; N, 4·1. $C_{17}H_{13}O_4NS$ requires C, 62·4; H, 4·0; N, 4·3%). The substance was prepared in lower yield by the method described for the previous compound.

Desulphurisation of N-Ethyl-α-carboxymethylthiosuccinimide.—The compound (1·0 g.), Raney nickel (" 15 c.c."), and alcohol (*ca.* 40 c.c.) were heated under reflux for 19 hours, N-sodium hydroxide added until the solution was alkaline to phenolphthalein, and the nickel filtered off through " Hyflo Supercel." The alcoholic solution was diluted with water, and the alcohol removed *in vacuo* at 60°. The residue was dissolved in a little water and continuously extracted with ether overnight. (*a*) The aqueous phase was acidified to Congo-red with 5N-sulphuric acid and, after addition of an equal volume of xylene, distilled, with the addition when necessary of equal volumes of water and xylene, until the distillate was no longer acid. 4·8 c.c. of N-sodium hydroxide was required to neutralise the distillate. The solution of the sodium salt was evaporated to dryness, dissolved in a little alcohol with the addition of a little water, clarified with charcoal, made just acid with hydrochloric acid, and heated under reflux with *p*-phenylphenacyl bromide (0·14 g.) for 2 hours. Some inorganic material separated on cooling, followed by the ester, m. p. 107—108° (40 mg.), undepressed on admixture with that of acetic acid. (*b*) The ethereal phase was dried (Na_2SO_4) and evaporated, and the residue hydrolysed by boiling 5N-sodium hydroxide (10 c.c.; 2 hours; basic fumes evolved). The aqueous solution was extracted thoroughly with ether, acidified with 10N-sulphuric acid, and extracted continuously with ether. The dried and evaporated extract gave 0·22 g. of yellow crystals which were identified, after recrystallisation from water, as succinic acid by m. p., mixed m. p., and by equivalent weight (Found: 59·9. Calc. for $C_4H_6O_4$: 59·0).

Desulphurisation of α-o-Carboxyphenyl-N-ethylthiosuccinimide.—The product of desulphurisation (performed exactly as above) was freed from the nickel by continuous extraction with hot alcohol and, after evaporation of the extract, partitioned between ether and aqueous potassium hydrogen carbonate. From the ether succinic acid was isolated after hydrolysis, and from the alkaline layer benzoic acid, which was purified by sublimation and identified by m. p. and mixed m. p.

Desulphurisation of N-Phenyl-α-carboxymethylthiosuccinimide.—This reaction was carried out as above. The ethereal extract eventually furnished a small yield of succinanil (Menschutkin, *Annalen*, 1872, **162**, 166), identified, after recrystallisation from alcohol, by its m. p. (151·5—152·5°), not depressed by an authentic specimen. The hydrogen carbonate phase gave a very slightly acid distillate with xylene, in agreement with the small yield of the other desulphurisation product.

Desulphurisation of N-Phenyl-α-o-carboxyphenylthiosuccinimide.—The reaction was carried out as above. The product was partitioned between chloroform and aqueous potassium hydrogen carbonate. From the former solvent was isolated succinanil, m. p. and mixed m. p. 151—153° after recrystallisation from alcohol, and from the latter benzoic acid, purified by sublimation, m. p. and mixed m. p. 119—120·5°.

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323. *Studies in the Polyene Series. Part XXVIII. The Structure of the C_{14} Aldehyde derived from β-Ionone and its Use for the Synthesis of Norvitamin A and* iso*Vitamin A Derivatives.*

By G. W. H. CHEESEMAN, SIR IAN HEILBRON, E. R. H. JONES, F. SONDHEIMER, and B. C. L. WEEDON.

Evidence for the structure of the C_{14} aldehyde, formed by application of the Darzens reaction to β-ionone, is reviewed. The conclusion is drawn that (I) is the only formulation compatible with the reactions of the aldehyde.

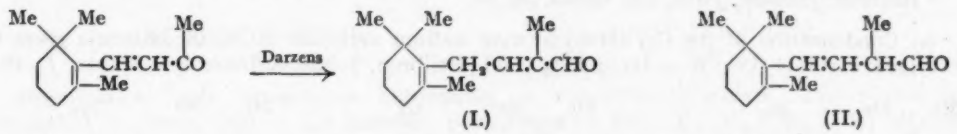
Condensation of (I) with 1-methoxypent-2-en-4-yne (X; $R_1 = H$, $R_2 = Me$) yields the methoxy-carbinol (XI; $R_1 = H$, $R_2 = Me$), which on partial hydrogenation gives the tetraene (XII; $R_1 = H$, $R_2 = Me$). The latter, on treatment with iodine, is converted into the norvitamin A methyl ether (XIII; $R_1 = H$, $R_2 = Me$). The *iso*vitamin A methyl ether (XIII; $R_1 = R_2 = Me$) is similarly prepared from the methoxy-carbinol (XI; $R_1 = R_2 = Me$).

By condensation of pent-2-en-4-yn-1-ol and hex-3-en-5-yn-2-ol with (I), the glycols (XI; $R_1 = R_2 = H$) and (XI; $R_1 = Me$, $R_2 = H$) are obtained. Partial hydrogenation of the former product yields the tetraene glycol (XII; $R_1 = R_2 = H$), the monoacetate of which, on treatment with iodine, gives the norvitamin A acetate (XIII; $R_1 = H$, $R_2 = Ac$). The product formed on partial hydrogenation of the diacetate of the glycol (XI; $R_1 = Me$, $R_2 = H$) yields, on treatment with iodine, the *iso*vitamin A acetate (XIII; $R_1 = Me$, $R_2 = Ac$) and a hydrocarbon, $C_{20}H_{28}$, exhibiting light absorption similar to that of anhydrovitamin A. [The nor- and *iso*-vitamin A derivatives described are obtained as concentrates containing 15—45% of the required polyenes.]

The norvitamin A methyl ether (XIII; $R_1 = H$, $R_2 = Me$) possesses definite growth promoting properties (not less than one-thirtieth of that of vitamin A) when fed orally to vitamin-A-deficient rats.

THE decarboxylation, in the presence of copper powder, of the glycide acid derived from β-ionone was described by Heilbron, Johnson, Jones, and Spinks (*J.*, 1942, 727) and shown to give the C_{14} aldehyde (I), which was purified by regeneration from the thiosemicarbazone or semicarbazone (Cymerman, Heilbron, Jones, and Lacey, *J.*, 1946, 500). Subsequently Isler, Huber, Ronco, and Kofler (*Helv. Chim. Acta*, 1947, **30**, 1911) found that, when the Darzens condensation of β-ionone with ethyl chloroacetate was carried out at −10° and the product hydrolysed at *ca.* +5° without isolation of the glycide ester, the pure C_{14} aldehyde (I) was obtained directly in 80% yield. We have confirmed this important observation and by comparison of their derivatives and reactions, established that the aldehydes prepared by the two routes are identical.

Recently, Milas *et al.* (*J. Amer. Chem. Soc.*, 1948, **70**, 1584; Milas, "Vitamins and Hormones," New York, 1947, **5**, 1) reported the decarboxylation of the pyridine salt of the glycide acid and concluded that the main product had the structure (II). In view of the fact that the



(I.) (II.)

C_{14} aldehyde is a key intermediate for the synthesis of vitamin A and related compounds (Isler *et al.*, *loc. cit.*; *Experientia*, 1946, **2**, 31; Emil Barell Jubilee Volume, Hoffmann–La Roche and Co., Ltd., Basel, 1946, p. 31; Heilbron, *J.*, 1948, 386; Milas *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1591, 1597), it is highly desirable that any remaining doubts as to its structure should be dispelled. Accordingly we summarise the existing evidence, which we regard as clearly indicating the structure (I), and at the same time advance new evidence as confirmation. It should be noted at the outset that in all our experiments we have employed only the pure aldehyde, either prepared directly by the method of the Swiss workers, or regenerated under mild conditions from a crystalline derivative. On the other hand, Milas *et al.* (*loc. cit.*) have used material, the homogeneity of which, on the evidence provided, is in our opinion extremely doubtful. Their crude decarboxylation product was twice fractionated through a 6-inch Vigreux column and the main fraction was used in all synthetic and structural studies since "it was felt that this, being the largest portion, represented the main product of the reaction."

(*a*) The light absorptions (see Table I) of the 2 : 4-dinitrophenylhydrazone, semicarbazone, thiosemicarbazone, and phenylsemicarbazone (Heilbron *et al.*, *loc. cit.*) are typical of those of αβ-unsaturated aldehydes. The formation of crystalline derivatives from the main fraction of Milas *et al.* "was slow and the yields were low." Whereas the 2 : 4-dinitrophenylhydrazone and semicarbazone described by these authors agree reasonably in melting point and light-absorption data with those prepared by us (in yields of *ca.* 80% from the pure aldehyde), the thiosemicarbazone, like that obtained previously by Ishikawa and Matsuura (*Sci. Rep. Tokyo Bunrika Daigaku, A*, 1937, **3**, 173; *Chem. Zentr.*, 1937, II, 3452), melts considerably lower (*ca.* 30—40°) than those prepared by us and by Isler *et al.* (Emil Barell Jubilee Volume). It is to be regretted that Milas *et al.* have not reported the light-absorption properties of their thiosemicarbazone.

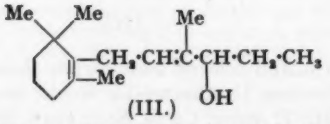
(*b*) Condensation of the C_{14} aldehyde with ethylmagnesium bromide furnishes a secondary *carbinol* (in 90% yield) which exhibits no light absorption of appreciable intensity in the region 2200—4000 A., in agreement with the formulation (III). On the other hand, Milas *et al.*, from their main fraction possessing $\varepsilon_{max.} = 20{,}500$ (2320 A.), obtained a product which had $\varepsilon_{max.} = 6000$ (2260 A.). A carbinol derived from an aldehyde of structure (II) must show light-absorption properties very similar (in both $\lambda_{max.}$ and $\varepsilon_{max.}$) to those of the original aldehyde.

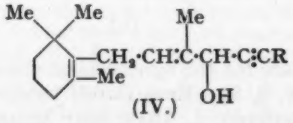
TABLE I.

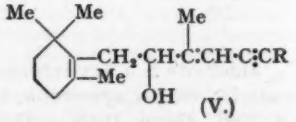
2 : 4-*Dinitrophenylhydrazone* ($CHCl_3$)	$\lambda_{max.}$ A	$\varepsilon_{max.}$
Of $CH_3 \cdot CH_2 \cdot CHO$ [1]	3610	21,000
Of citral [1] (>C=CH·CHO)	3850	31,000
Of C_{14} aldehyde [1] (–CH=C(Me)·CHO)	3830	29,500
Semicarbazone.		
Of *iso*pulegone [2] (CH_2=C(Me)·CH·CO)	<2400	—
Of citral [3] (>C=CH·CHO)	2720	31,500
Of C_{14} aldehyde (–CH=C(Me)·CHO)	2650 2690	29,500 29,000
Thiosemicarbazone.		
Of $CH_3 \cdot CH_2 \cdot CHO$ [4]	2700	20,500
Of α-ionone [4] (–CH=CH·C(Me)O)	3025	36,500
Of C_{14} aldehyde [5] (–CH=C(Me)·CHO)	2990	39,000
Phenylsemicarbazone.		
Of *β-cyclo*citral [3] (>C=C(Me)·CHO)	2805	26,500
Of C_{14} aldehyde [5] (–CH=C(Me)·CHO)	2780	37,000

[1] Braude and Jones, *J.*, 1945, 498. [2] Menschick, Page, and Bossert, *Annalen*, 1932, **495**, 225.
[3] Burawoy, *J.*, 1941, 20. [4] Evans and Gillam, *J.*, 1943, 565.
[5] Heilbron, Johnson, Jones, and Spinks, *loc. cit.*

(*c*) Condensation of the C_{14} aldehyde with sodium acetylide in liquid ammonia gives the acetylenic carbinol (IV; R = H) in 33% yield (Heilbron, Johnson, Jones, and Spinks, *J.*, 1942,

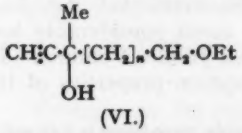

(III.)

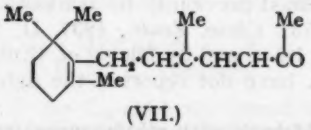

(IV.)

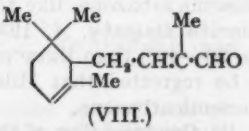

(V.)

727), and by the use of lithium acetylide the yield of this substance has now been increased to 75%. Condensation with hexynylmagnesium bromide furnishes the carbinol (IV; R = Bu^n) (Cymerman, Heilbron, Jones, and Lacey, *loc. cit.*); neither of these carbinols shows light absorption of appreciable intensity in the ultra-violet. When the carbinol (IV; R = Bu^n) is shaken with dilute sulphuric acid, the rearranged carbinol (V; R = Bu^n) is obtained in nearly quantitative yield. The latter shows the typical light-absorption properties ($\lambda_{max.}$ = 2290 A.) of a conjugated enyne. Again, the carbinol (IV; R = CH:CH·CHMe·OMe) on shaking with dilute acid gives (V; R = CH:CH·CHMe·OMe) which shows the characteristic light-absorption properties of a conjugated dienyne (*idem*, *loc. cit.*). The very occurrence of these anionotropic rearrangements, and the light-absorption properties of the products, can only be interpreted if the parent C_{14} aldehyde is αβ-unsaturated.

Milas *et al.* condensed their main fraction with acetylene (*loc. cit.*, p. 1584) and also with the ethynylcarbinols (VI; *n* = 1 and 2) (*loc. cit.*, p. 1591, 1597). None of the products exhibited absorption at 2200—2260 A. of intensity ($\varepsilon_{max.}$ = 9500, 4470, and 9800, respectively) comparable with that of the starting material ($\varepsilon_{max.}$ = 20,500), as would have been the case had the latter consisted of an aldehyde of structure (II).


(VI.)


(VII.)


(VIII.)

(*d*) Condensation of the C_{14} aldehyde with acetone under Oppenauer conditions (*cf.* Heilbron, Johnson, Jones, and Spinks, *loc. cit.*) has now provided the ketone (VII) as a crystalline solid. Its light-absorption properties are typical of those of a doubly unsaturated ketone. In this instance, however, the possibility of migration of a double bond under the experimental conditions cannot be definitely excluded.

(*e*) That no migration of the cyclic double bond occurs during the formation of the C_{14} aldehyde (I) from β-ionone is shown by the fact that an entirely different, isomeric, αβ-unsaturated aldehyde (VIII) is obtained by decarboxylation of the glycide acid from α-ionone (*idem, loc. cit.*). Further, the successful application of (I) in the preparation of vitamin A (Isler *et al.*, *loc. cit.*) and related polyenes (see below) provides additional confirmation that the position of the cyclic double bond has been correctly assigned.

(*f*) Milas *et al.* (*loc. cit.*, p. 1584), in support of formula (II) for their main fraction, stated that ozonolysis by the method of Strain (*J. Biol. Chem.*, 1933, **102**, 137) gave geronic acid in 40% yield. We have ozonised the pure aldehyde (I), also following the method of Strain, and failed to detect any geronic acid. Working under identical conditions we obtained geronic acid in 45% yield from β-ionone.

We have always realised that the crude product from the copper-bronze decarboxylation of the glycide acid is a mixture, quite probably of oxide and aldehydes, and for this reason we have used either the regenerated C_{14} aldehyde or, latterly, the aldehyde made according to Isler *et al.* (*Helv. Chim. Acta*, 1947, **30**, 1911). On the basis of the evidence obtained by us and submitted above, we are convinced that only the structure (I) is compatible with the reactions of the pure C_{14} aldehyde prepared by either of the two routes described.†

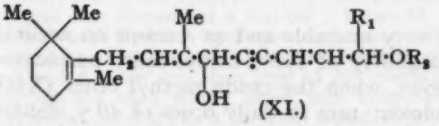
As part of an extensive study of the relations between chemical constitution and biological activity in the vitamin A field (*vide* Heilbron, *J.*, 1948, 386; Heilbron, Jones, and Richardson, this vol., p. 287), we have synthesised the acetate and methyl ether of both the norvitamin A (XIII; $R_1 = R_2 = H$) and the *iso*vitamin A (XIII; $R_1 = Me$, $R_2 = H$). The procedures employed were essentially those envisaged by Heilbron *et al.* (*J.*, 1942, 727; 1944, 141) and used later by Isler *et al.* (*loc. cit.*) for the preparation of vitamin A (XIV) and its methyl ether.

$HC{\equiv}C{\cdot}CH(OH){\cdot}CH{:}CH_2$ (IX.)

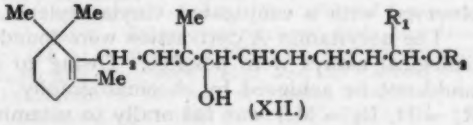
$HC{\equiv}C{\cdot}CH{:}CH{\cdot}\underset{R_1}{CH}{\cdot}OR_2$ (X.)

Bromination, with phosphorus tribromide, of vinylethynylcarbinol (IX) (Jones and McCombie, *J.*, 1942, 733) or the isomeric pent-2-en-4-yn-1-ol (X; $R_1 = R_2 = H$) (Haynes, Heilbron, Jones, and Sondheimer, *J.*, 1947, 1583), and treatment of the resulting crude bromide with sodium methoxide yielded (55 and 65%, respectively) *1-methoxypent-2-en-4-yne* (X; $R_1 = H$, $R_2 = Me$). Condensation of the Grignard complex of this ether with the C_{14} aldehyde (I) gave the *methoxycarbinol* (XI; $R_1 = H$, $R_2 = Me$) in 75% yield. This compound, containing a conjugated vinylacetylene chromophore, was partly hydrogenated in the presence of a quinoline-poisoned palladium–charcoal catalyst (for a gift of which the authors are indebted to Messrs. Hoffmann–La Roche and Co., Ltd., Basel). The resulting crude tetraene (XII; $R_1 = H$, $R_2 = Me$), on being heated with a trace of iodine in toluene solution, underwent simultaneous rearrangement and dehydration yielding the norvitamin A methyl ether (XIII; $R_1 = H$, $R_2 = Me$). Similarly, the methoxy-carbinol (XI; $R_1 = R_2 = Me$) (Cymerman, Heilbron, Jones, and Lacey, *loc. cit.*) gave the *iso*vitamin A methyl ether (XIII; $R_1 = R_2 = Me$). The intensity of maximal light absorption in the region 3230—3280 A. indicated that the reaction products contained *ca.* 45 and 40%, respectively, of the ethers.

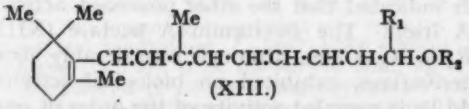
Condensation of the di-Grignard complex of both pent-2-en-4-yn-1-ol (X; $R_1 = R_2 = H$) and hex-3-en-5-yn-2-ol (X; $R_1 = Me$, $R_2 = H$) (Heilbron, Jones, Smith, and Weedon, *J.*, 1946,



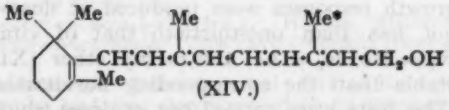
(XI.)



(XII.)



(XIII.)



(XIV.)

54) with the C_{14} aldehyde (I) gave (60 and 55%) the *glycols* (XI; $R_1 = R_2 = H$) and (XI; $R_1 = Me$, $R_2 = H$), respectively. Partial hydrogenation of (XI; $R_1 = R_2 = H$) yielded a

† *Added in Proof.*—Since this paper was written, Inhoffen *et al.* (*Annalen*, 1948, **561**, 26) have reported that the Raman spectrum of the C_{14} aldehyde both supports the structure (I) and disproves the formula (II) favoured by Milas.

mixture consisting essentially of the polyene *glycol* (XII; $R_1 = R_2 = H$). The primary hydroxyl group was protected by selective acetylation with acetyl chloride and pyridine, and the *monoacetate* (XII; $R_1 = H$, $R_2 = Ac$) thus formed was heated with a trace of iodine in light petroleum; from the intensity of the light-absorption maximum at 3240 A., the product was estimated to contain *ca.* 25% of the norvitamin A acetate (XIII; $R_1 = H$, $R_2 = Ac$).

Acetylation of the glycol (XI; $R_1 = Me$, $R_2 = H$) with acetic anhydride and pyridine gave the corresponding *diacetate*; this was partly hydrogenated, and the resulting crude tetraene heated with a trace of iodine in toluene. The product, exhibiting a light-absorption maximum at 3260 A., was estimated to contain *ca.* 15% of the *iso*vitamin A acetate (XIII; $R_1 = Me$, $R_2 = Ac$).

Iodine treatment of the diacetate of (XII; $R_1 = Me$, $R_2 = H$) was found frequently to give rise, not only to the required polyene acetate (XIII; $R_1 = Me$, $R_2 = Ac$), but also, by loss of two molecules of acetic acid, to an unstable *hydrocarbon* which was readily isolated by chromatography and exhibited light absorption similar to that of anhydrovitamin A (Table II).

TABLE II.

	$\lambda_{max.}$ A.	$\varepsilon_{max.}$
$HC{\vdots}C\cdot CH{:}CH\cdot CH_2\cdot OMe$	2220	12,500
	2290 *	11,500
$HC{\vdots}C\cdot CH{:}CH\cdot CHMe\cdot OMe$ [1]	2235	12,500
	2320 *	11,000
$HO\cdot CH_2\cdot C{\vdots}C\cdot CH{:}CH\cdot CH_2\cdot OH$ [2]	2270	15,000
	2370 *	12,000
(XI; $R_1 = H$, $R_2 = Me$)	2290	17,500
	2370 *	15,000
(XI; $R_1 = R_2 = H$)	2280	16,000
	2380 *	13,000
(XI; $R_1 = R_2 = Me$) [3]	2235	16,000
	2270	16,000
	2350 *	13,500
(XI; $R_1 = Me$, $R_2 = H$)	2280	13,500
	2410 *	10,500
Diacetate of (XI; $R_1 = Me$, $R_2 = H$)	2280	15,000
Hydrocarbon, $C_{20}H_{28}$	3510	46,500
	3660	55,000
	3840	40,000
Anhydrovitamin A [4]	3520	51,000
	3700	78,000
	3910	70,000

* Inflexion.
[1] Heilbron, Jones, and Weedon, *J.*, 1945, 81. [2] Heilbron, Jones, and Sondheimer, *J.*, 1947, 1586.
[3] Cymerman, Heilbron, Jones, and Lacey, *loc. cit.*
[4] Isler, Huber, Ronco, and Kofler, *Helv. Chim. Acta*, 1947, **30**, 1911.

This hydrocarbon was also obtained on iodine treatment of the glycol (XII; $R_1 = Me$, $R_2 = H$), prepared by partial hydrogenation of (XI; $R_1 = Me$, $R_2 = H$).

The various intermediates in the polyene syntheses described above exhibited the expected light-absorption properties. The glycols (XI; $R_1 = H$ and Me, $R_2 = H$) and the methoxycarbinols (XI; $R_1 = H$ and Me, $R_2 = Me$) all showed the characteristic maximum and inflexion observed with a conjugated vinylacetylene.

The norvitamin A derivatives were found to be very unstable and as a result no accurate biological assays were possible. Owing to the instability of these compounds, purification could not be achieved by chromatography. However, when the crude methyl ether (XIII; $R_1 = H$, $R_2 = Me$) was fed orally to vitamin-A-deficient rats in daily doses of 40 γ, definite growth responses were produced at doses which indicated that the ether possessed activity not less than one-thirtieth that of vitamin A itself. The *iso*vitamin A acetate (XIII; $R_1 = Me$, $R_2 = Ac$) and methyl ether (XIII; $R_1 = R_2 = Me$), although considerably more stable than the corresponding norvitamin A derivatives, exhibited no biological activity. (The tests were carried out at doses which would have revealed activity of the order of one-hundredth and one-thousandth, respectively, of that of vitamin A.) It may be concluded that the presence of the $C_{(3)}$-methyl group (Me*) in the polyene chain of vitamin A (XIV), although contributing very appreciably to the stability of the molecule, is not essential for biological activity.

Experimental.

Light-absorption data were determined in ethanol, except where stated otherwise. The purities of the polyene concentrates were estimated on the assumptions that absorption in the region 3230—3280 A. was due entirely to the nor- and *iso*-vitamin A derivatives, and that the latter, when pure, would exhibit absorption of the same intensity as vitamin A. All the operations were carried out in an atmosphere of nitrogen.

γ-(2 : 6 : 6-*Trimethyl*cyclo*hex*-1-*enyl*)-*α*-*methylcrotonaldehyde* (C_{14} *Aldehyde*) (I).—Sodium methoxide (38 g.; commercial) was added in portions, during 30 minutes, to a well-stirred mixture of ethyl chloroacetate (80 g.) and *β*-ionone (100 g.; light absorption: maxima, 2230, 2960 A.; ε, 6,500 and 10,000 respectively), the temperature being maintained at −15° by means of an alcohol–carbon dioxide bath. Stirring was continued for 4 hours at −10°, and then a cold solution of sodium hydroxide (45 g.) in water (50 c.c.) and methanol (250 c.c.) was added during 30 minutes, the temperature being kept at 0°. After the mixture had been stirred for a further 2 hours at 5°, water was added and the product was isolated by means of ether and distilled. The fraction (87 g.), b. p. 110—125°/0·3 mm., was carefully redistilled to give the aldehyde (71 g.), b. p. 91—92°/0·015 mm., $n_D^{19°}$ 1·5113 (light absorption: maximum, 2290 A.; ε, 16,000). The semicarbazone had m. p. 156—157° (Cymerman, Heilbron, Jones, and Lacey, *J.*, 1946, 500, give m. p. 148—150°; Milas *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1584, give m. p. 149·5—150·5°); the 2 : 4-dinitrophenylhydrazone (obtained in 80% yield) had m. p. 173° (Heilbron, Johnson, Jones, and Spinks, *J.*, 1942, 727 give m. p. 164·5°; Milas *et al.*, *loc. cit.*, give m. p. 169—170°); the thiosemicarbazone (obtained in 80% yield) had m. p. 200°, undepressed on admixture with a specimen (m. p. 200°) prepared by the method of Heilbron *et al.* (*loc. cit.*) [Isler *et al.*, Emil Barell Jubilee Volume, Hoffmann–La Roche and Co., Ltd., Basel, 1946, p. 31, give m. p. 193—194° (corr.); Ishikawa and Matsuura, *loc. cit.*, give m. p. 160° (corr.); Milas *et al.*, *loc. cit.*, give m. p. 156—159°]. A sample of C_{14} aldehyde, regenerated from the thiosemicarbazone by steam-distillation in the presence of oxalic acid, had $n_D^{19°}$ 1·5120. Light-absorption maximum: 2300 A.; ε, 17,500.

6-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-4-*methylhex*-4-*en*-3-*ol* (III).—The C_{14} aldehyde (10·3 g.) in ether (100 c.c.) was added gradually, with stirring, to a solution of ethylmagnesium bromide (prepared from 1·8 g. of magnesium) in ether (100 c.c.), and the mixture was boiled under reflux for 3 hours. Decomposition of the Grignard complex with aqueous ammonium chloride, and isolation of the product with ether, yielded the *carbinol* (10·5 g.) as a viscous liquid, b. p. 101°/0·05 mm., $n_D^{19°}$ 1·5012 (Found: C, 81·1; H, 11·85. $C_{16}H_{28}O$ requires C, 81·3; H, 11·95%). The carbinol showed no light absorption of appreciable intensity at >2200 A.

6-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-4-*methylhex*-4-*en*-1-*yn*-3-*ol* (IV; R = H).—A rapid stream of dry acetylene was passed through a stirred suspension of lithium (0·7 g.), in small pieces, in liquid ammonia (400 c.c.), cooled in a carbon dioxide–alcohol bath. When all the lithium had reacted, C_{14} aldehyde (12 g.) in ether (50 c.c.) was added dropwise and the mixture was stirred overnight, a slow stream of acetylene being passed in throughout. Ammonium chloride (10 g.) was added, the ammonia was evaporated on the steam-bath, and the residue was extracted thoroughly with ether. The ethereal extract was washed with water, dried, and evaporated. Distillation of the residue then gave the acetylenic carbinol (10·1 g.) as a pale yellow oil, b. p. 120—122°/0·2 mm., $n_D^{20°}$ 1·5130 (Found: C, 82·6; H, 10·5. Calc. for $C_{16}H_{24}O$: C, 82·8; H, 10·35%). The carbinol showed no light absorption of appreciable intensity at >2200 A. It formed a white silver derivative with ammoniacal silver nitrate.

7-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-5-*methylhepta*-3 : 5-*dien*-2-*one* (VII).—A solution of C_{14} aldehyde (10 g.) and freshly prepared aluminium *tert.*-butoxide (12 g.) in benzene (300 c.c.) and acetone (200 c.c.) was heated under reflux for 48 hours. After cooling it, 10% (w/v) sulphuric acid was added and the organic layer was separated, washed well with water, dried, and evaporated. Distillation of the residue gave the ketone (3·6 g.), b. p. 118—122°/10^{-3} mm., which rapidly solidified. Crystallisation from pentane and then from aqueous methanol gave colourless prisms, m. p. 67—68° (Found: C, 82·5; H, 10·5. Calc. for $C_{17}H_{26}O$: C, 82·9; H, 10·6%) (light absorption: maxima, 2810 and 2900 A.; ε, 30,500 and 25,500, respectively). [*ψ*-Ionone, having the same chromophoric system, has light absorption: maximum, 2910 A.; ε, 22,000 (Burawoy, *J.*, 1941, 20).] The 2 : 4-*dinitrophenylhydrazone* crystallised from ethanol in dark red plates, m. p. 174—175° (Found: N, 13·2. $C_{23}H_{30}O_4N_4$ requires N, 13·15%) [light absorption in chloroform (main band only): maximum, 3990 A.; ε, 33,000].

1-*Methoxypent*-2-*en*-4-*yne* (X; R_1 = H, R_2 = Me).—(*a*) Phosphorus tribromide (45 g.) in dry ether (30 c.c.) was added to a stirred and cooled solution of vinylethynylcarbinol (24·6 g.; Jones and McCombie, *J.*, 1942, 733) in dry ether (100 c.c.) during 15 minutes, the temperature being kept at −10°. The reaction mixture was stirred for a further 2 hours at −10° and then poured on ice. The ethereal layer was washed with sodium hydrogen carbonate solution and water. Ether was removed from the dried extract, the residue was added to a solution of sodium (14 g.) in methanol (200 c.c.), and the mixture was heated to 50° and then kept at 20° overnight. Water and ether were added, and the ethereal layer was washed with water, dried, and evaporated. Distillation of the residue yielded 1-*methoxypent*-2-*en*-4-*yne* (15·8 g.) as a pungent liquid, b. p. 80—82°/160 mm., $n_D^{18°}$ 1·4637 (Found: C, 74·75; H, 8·6. C_6H_8O requires C, 74·95; H, 8·4%) (light absorption: see Table II).

(*b*) The foregoing experiment was repeated using pent-2-en-4-yn-1-ol (24·6 g.; Haynes, Heilbron, Jones, and Sondheimer, *J.*, 1947, 1583) instead of vinylethynylcarbinol. This gave 1-methoxypent-2-en-4-yne (19·2 g.), b. p. 58—60°/70 mm., $n_D^{22°}$ 1·4603.

9-*Methoxy*-1-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-3-*methylnona*-2 : 7-*dien*-5-*yn*-4-*ol* (XI; R_1 = H, R_2 = Me).—1-Methoxypent-2-en-4-yne (11 g.) in ether (50 c.c.) was slowly added to an ice-cooled solution of ethylmagnesium bromide (from 2·6 g. magnesium) in ether (150 c.c.), and the mixture was heated under reflux for 2 hours. After cooling to 0°, C_{14} aldehyde (11·7 g.) in ether (50 c.c.) was added dropwise. Stirring was continued overnight (15 hours), and the reaction was completed by boiling under reflux for a further 2 hours. The cold mixture was decomposed with ammonium chloride solution, and the ethereal layer was washed with water, dried, and evaporated. Distillation of the residue yielded the *methoxy-carbinol* (13 g.) as a rather viscous yellow liquid, b. p. (bath temp.)

120—130°/10^{-4} mm., $n_D^{20°}$ 1·5290 (Found: C, 78·95; H, 10·1. $C_{20}H_{30}O$ requires C, 79·4; H, 10·0%) (light absorption: see Table II).

1-*Methoxy*-9-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-7-*methylnona*-2 : 4 : 6 : 8-*tetraene* (XIII; R_1 = H, R_2 = Me).—The above methoxy-carbinol (6·25 g.) in methanol (50 c.c.) was shaken in hydrogen in the presence of a palladium–charcoal catalyst (1 g., containing 0·5 g. of quinoline and 4% Pd) until 513 c.c. of gas had been absorbed at 19°/752 mm. (equivalent to 1 double bond). The catalyst was filtered off, the solvent was removed under diminished pressure, and the residue (6·3 g.; $n_D^{20°}$ 1·5216) was dissolved in toluene (120 c.c.). This solution was heated to 95°, and iodine (90 mg.) in toluene (30 c.c.) was added during 5 minutes with stirring. Heating at 95° was continued for a further 20 minutes. After cooling, the solution was washed with, successively, sodium thiosulphate solution, dilute sulphuric acid, sodium hydrogen carbonate solution, and water. The toluene was removed under reduced pressure, the residue was dissolved in light petroleum (240 c.c.; b. p. 40—60°), and the solution was washed 6 times with 95% aqueous methanol (6 × 60 c.c.). The combined methanol extracts were shaken twice with light petroleum, and the combined petroleum solutions were washed with water, dried, and evaporated. The residue (2·6 g.) was a yellow viscous liquid, $n_D^{20°}$ 1·577. The light absorption (maximum, 3230 A.; $E^{1\%}_{1\,cm.}$, 720) corresponds to *ca.* 45% content of the ether (XIII; R_1 = H, R_2 = Me). The product, which gave a dark-blue colour with chloroformic antimony trichloride, was unstable, and decomposed on attempted purification by chromatography on alumina.

9-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-7-*methylnona*-2 : 7-*dien*-4-*yne*-1 : 6-*diol* (XI; R_1 = R_2 = H).—A solution of ethylmagnesium bromide was prepared from magnesium (11·2 g.; 0·46 mole) in ether (200 c.c.). Benzene (300 c.c.) was added slowly, and at the same time the ether was distilled off. When the temperature of the escaping vapours had reached 70°, the mixture was cooled in ice, pent-2-en-4-yn-1-ol (18 g.; 0·22 mole) in benzene (50 c.c.) was added slowly, and the mixture was stirred for another hour, during which time a fine grey powder separated. The C_{14} aldehyde (I) (30 g.; 0·15 mole) in benzene (50 c.c.) was added dropwise to the ice-cooled suspension, which was then stirred for another 14 hours at room temperature and for 1 hour under reflux. After cooling, the reaction mixture was decomposed with aqueous ammonium chloride, and the organic layer was washed thoroughly with water and dried. The solvent was evaporated under reduced pressure; unchanged starting materials were removed by heating at 10^{-3} mm. The residue (34·6 g.) was suspended in light petroleum (b. p. 40—60°; 380 c.c.) and extracted four times with aqueous methanol, (75%; 4 × 120 c.c.) whereupon most of the dark red colour remained in the petroleum layer. The light-yellow methanol extracts were diluted with water, and the glycol was extracted with ether. The dried ethereal extract was evaporated and the residue was heated at 100°/10^{-4} mm., to give the *diol* (25·2 g.) as a light orange, very viscous oil, $n_D^{18°}$ 1·5437 (light absorption: maximum, 2290 A.; ε, 14,500; inflexion, 2370 A.; ε, 12,500). A small sample was distilled for analysis at 120—130° (bath temp.)/10^{-5} mm., whereafter the product had $n_D^{19°}$ 1·5460 (Found: C, 78·9; H, 9·7. $C_{19}H_{32}O_2$ requires C, 79·1; H, 9·8%) (light absorption; see Table II).

When the Grignard condensation was carried out in ethereal solution the yield of glycol was only 35%.

9-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-7-*methylnona*-2 : 4 : 7-*triene*-1 : 6-*diol* (XII; R_1 = R_2 = H).—The above diol (13·0 g.) in methanol (70 c.c.) was shaken in hydrogen in the presence of a partly-poisoned palladium–charcoal catalyst (1·4 g.; containing 0·7 g. of quinoline and 4% of Pd). The absorption of hydrogen was rather slow, and more catalyst (0·7 g.) was added during the hydrogenation, which was interrupted when 1130 c.c. of gas had been absorbed at 19°/768 mm. (equivalent to 1·03 double bonds). After removal of catalyst and solvent, water and ether were added and the ethereal layer was shaken with, successively, dilute acetic acid (until free from quinoline), sodium hydrogen carbonate solution, and water. The extract was dried and evaporated, giving the *diol* (12·6 g.), $n_D^{18°}$ 1·5348 [light absorption: maxima, 2310, 2710; ε, 13,500 and 4,500, respectively (the second maximum, probably due to a small amount of rearranged material, was observed in every case in which this experiment was carried out]. A small amount was distilled for analysis at 130—140° (bath temp.)/10^{-5} mm., $n_D^{19°}$ 1·5340 (Found: C, 78·35; H, 10·1. $C_{19}H_{30}O_2$ requires C, 78·55; H, 10·4%).

9-*Acetoxy*-1-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-3-*methylnona*-2 : 5 : 7-*trien*-4-*ol* (XII; R_1 = H, R_2 = Ac).—The preceding diol (11·7 g.) was dissolved in dry benzene (60 c.c.) and pyridine (60 c.c.), and a solution of acetyl chloride (3·5 g.) in benzene (60 c.c.) was added during 10 minutes with stirring and cooling. The reaction mixture was stirred for a further 14 hours at room temperature and then decomposed with ice. Ether was added and the organic layer was freed from pyridine by shaking with dilute acetic acid. After being washed with sodium hydrogen carbonate solution and water, the ethereal solution was dried and evaporated, finally at 100°/10^{-4} mm., giving the *monoacetate* (12·9 g.) as a viscous liquid, $n_D^{15°}$ 1·5210; distillation was not attempted (Found: C, 75·0; H, 9·65. $C_{21}H_{32}O_3$ requires C, 75·85; H, 9·7%) (light-absorption: maximum, 2340 A.; ε, 11,500: inflexion, 2680 A.; ε, 5500). With chloroformic antimony trichloride it gave a deep violet colour.

1-*Acetoxy*-9-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-7-*methyl*-2 : 4 : 6 : 8-*tetraene* (XIII; R_1 = H, R_2 = Ac).—A solution of the above monoacetate (12 g.) and a trace of quinol in light petroleum (b. p. 80—100°; 210 c.c.) was boiled under reflux. Iodine (120 mg.) in light petroleum (b. p. 80—100°; 30 c.c.) was added to the stirred solution during 5 minutes, and the reaction mixture was heated under reflux for a further 40 minutes. The cooled solution was washed once with sodium thiosulphate solution and five times with aqueous methanol (95%; 5 × 100 c.c.). The combined methanol extracts were once more washed with light petroleum, and the combined petroleum extracts were washed with water, dried, and evaporated under reduced pressure. The residue (7·6 g.) was an orange, rather viscous liquid, $n_D^{12°}$ 1·5527. The light absorption (maxima, 2290, 2430, 2810, and 3240 A.; $E^{1\%}_{1\,cm.}$, 270, 270, 420, and 400, respectively; inflexion, 3150 A.; $E^{1\%}_{1\,cm.}$, 350) corresponds to a *ca.* 25% content of the norvitamin A acetate (XIII; R_1 = H, R_2 = Ac). With chloroformic antimony trichloride it gave a bluish-violet colour. The acetate was unstable and decomposed on attempted purification by chromatography on alumina. When the iodine treatment was carried out for only 15 minutes, the product had light-absorption maxima (2410, 2790, 3240 A.; $E^{1\%}_{1\,cm.}$, 290, 370, and 240, respectively), corresponding to a *ca.* 15% content of the norvitamin acetate.

9-Methoxy-1-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-3-*methyldeca*-2 : 7-*dien*-5-*yn*-4-*ol* (XI; R_1 = R_2 = Me).—This was prepared by the method of Cymerman, Heilbron, Jones, and Lacey (*J.*, 1946, 500) and had b. p. 90—100° (bath temp.)/10^{-4} mm., $n_D^{18°}$ 1·5332 (light-absorption: maxima, 2280 and 3080 A.; ε, 19,500 and 3,000, respectively) [*idem*, *loc. cit.*, give b. p. 94—98° (bath temp.)/10^{-4} mm., $n_D^{17°}$ 1·5300, and maxima at 2235 and 2270 A.; ε, 16,000 in each case, and an inflexion at 2350 A.; ε, 13,500].

2-*Methoxy*-10-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-8-*methyldeca*-3 : 5 : 7 : 9-*tetraene* (XIII; R_1 = R_2 = Me).—The preceding methoxy-carbinol (4·8 g.) in methanol (40 c.c.) was shaken in hydrogen in the presence of a partly-poisoned palladium–charcoal catalyst (0·4 g.; containing 0·2 g. of quinoline and 4% Pd) until 359 c.c. of gas had been absorbed at 18°/769 mm. (equivalent to 1 double bond). Removal of the catalyst and evaporation of the solvent under diminished pressure gave crude 2-methoxy-10-(2′ : 6′ : 6′-trimethyl*cyclo*hex-1′-enyl)-8-methyldeca-3 : 5 : 8-trien-7-ol (4·8 g.) as an oil (light absorption: maxima, 2270 and 2910 A.; $E^{1\%}_{1\,cm.}$, 500 and 100, respectively).

A solution of the hydrogenation product (2 g.) and a trace of quinol in toluene (40 c.c.) was heated to 95°. Iodine (30 mg.) in toluene (10 c.c.) was added to the stirred solution during 5 minutes, and the reaction mixture was maintained at 95° for a further 20 minutes. After cooling, the toluene solution was washed with a solution of sodium thiosulphate and water, dried, and evaporated under reduced pressure. The residue was dissolved in light petroleum (b. p. 40—60°; 80 c.c.), and the solution was washed five times with aqueous methanol (95%; 5 × 20 c.c.). The combined methanol extracts were washed once more with light petroleum, and the combined petroleum solutions were washed with water, dried, and evaporated under reduced pressure, giving a dark-yellow viscous liquid (1·3 g.), $n_D^{21°}$ 1·5903. The light absorption (maxima, 3280 and 3830 A.; $E^{1\%}_{1\,cm.}$, 630 and 290, respectively) corresponds to a *ca.* 40% content of the methyl ether (XIII; R_1 = R_2 = Me). In view of the biological inactivity of this material, further purification was not attempted.

10-(2′ : 6′ : 6′-*Trimethyl*cyclo*hex*-1′-*enyl*)-8-*methyldeca*-3 : 8-*dien*-5-*yne*-2 : 7-*diol* (XI; R_1 = Me, R_2 = H).—A solution of ethylmagnesium bromide was prepared from magnesium (9·5 g.; 0·39 mole) in ether (300 c.c.). Benzene (200 c.c.) was added slowly and at the same time the ether was distilled off. When the temperature of the escaping vapours had reached 70°, the solution was cooled to 0° and hex-3-en-5-yn-2-ol (17·5 g.; 0·18 mole; Heilbron, Jones, Smith, and Weedon, *J.*, 1946, 54) in benzene (100 c.c.) was added during 30 minutes. After the mixture had been stirred for a further 5 hours at 20° and the resulting suspension had been cooled to 0°, a solution of C_{14} aldehyde (I) (25 g.; 0·12 mole) in benzene (75 c.c.) was added during 30 minutes, and the mixture was then stirred at 20° for 24 hours. The complex was decomposed by adding aqueous ammonium chloride, and the organic layer was separated, washed with water, dried, and evaporated. Hex-3-en-5-yn-2-ol (5·9 g.) was recovered by distillation at 25 mm., and the residue (31 g.) was suspended in light petroleum (b. p. 40—60°; 250 c.c.). The solution was extracted four times with aqueous methanol (80%; 4 × 100 c.c.), and the combined extracts were washed once with light petroleum (b. p. 40—60°) and then diluted with water. Isolation with ether in the usual manner gave the *diol* (20·7 g.) as a yellow viscous oil, $n_D^{20°}$ 1·5342 (light absorption: see Table II). A small portion was distilled for analysis; b. p. 105—120° (bath temp.)/10^{-4} mm., $n_D^{16°}$ 1·5380 (Found: C, 79·0; H, 10·0. $C_{20}H_{30}O_2$ requires C, 79·4; H, 10·0%).

2 : 7-*Diacetoxy*-10-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-8-*methyldeca*-3 : 8-*dien*-5-*yne*.—Acetic anhydride (13·6 g.) was slowly added to a solution of the foregoing diol (16·7 g.) in pyridine (75 c.c.), and the mixture was set aside at 20° for 24 hours. Water was then added and the product (17 g.) was isolated in the usual way. Distillation gave the *diacetate* (14 g.) as a pale yellow viscous oil, b. p. 120—140° (bath temp.)/10^{-3} mm., $n_D^{20°}$ 1·5070 (Found: C, 74·25; H, 8·9. $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·8%) (light absorption: see Table II).

2-*Acetoxy*-10-(2′ : 6′ : 6′-*trimethyl*cyclo*hex*-1′-*enyl*)-8-*methyldeca*-3 : 5 : 7 : 9-*tetraene* (XIII; R_1 = Me, R_2 = Ac).—The preceding diacetate (13·7 g.) in methanol (30 c.c.) was shaken in hydrogen in the presence of a partly-poisoned palladium–charcoal catalyst (2 g.; containing 1 g. of quinoline and 4% of Pd) until 890 c.c. of gas had been absorbed at 21°/754 mm. (equivalent to 1 double bond). Removal of catalyst and solvent gave the crude product (13 g.), $n_D^{22°}$ 1·5090 (light absorption: maxima, 2280 and 2790 A.; $E^{1\%}_{1\,cm.}$, 400 and 190, respectively).

The partly hydrogenated diacetate (0·55 g.) and a trace of quinol were dissolved in toluene (10 c.c.), and the solution was heated to 95°. Iodine (10 mg.) in toluene (10 c.c.) was added with stirring during 5 minutes, and the solution was maintained at 95—100° for a further 90 minutes. After cooling, the toluene solution was washed with, successively, sodium thiosulphate solution, 10% (w/v) sulphuric acid, sodium hydrogen carbonate solution, and water. The solution was dried and then evaporated under reduced pressure, giving a yellow viscous oil (0·5 g.) which gave a dark blue colour with chloroformic antimony trichloride. It had light absorption: maxima at 2230 and 3260 A.; $E^{1\%}_{1\,cm.}$, 350 and 270, respectively: inflexion at 2820 A.; $E^{1\%}_{1\,cm.}$, 230, which corresponds to a *ca.* 15% content of the acetate (XIII; R_1 = Me, R_2 = Ac).

The Hydrocarbon, $C_{20}H_{28}$.—The partly hydrogenated diacetate (11·8 g.) was subjected to iodine treatment, in the manner described above, and the crude product (11·5 g.) (light absorption: maxima, 2270, 2790, 3230, 3450, 3620, and 3840 A.; $E^{1\%}_{1\,cm.}$, 300, 250, 220, 270, 270, and 175, respectively) was dissolved in light petroleum (b. p. 40—60°; 100 c.c.); the solution was filtered through a column of partly deactivated alumina (800 g.; grade III—IV; cf. Brockmann and Schodder, *Ber.*, 1941, **74**, 73). The chromatogram was developed with light petroleum (b. p. 40—60°; 900 c.c.), and the bright-yellow, clearly defined, and least-strongly adsorbed zone was removed and eluted with acetone. Evaporation of the solvent under reduced pressure gave an oil (1·1 g.) (light absorption: maxima, 3500, 3640, and 3840 A.; $E^{1\%}_{1\,cm.}$, 1150, 1250, and 1000, respectively) which was distilled; b. p. 80—100° (bath temp.)/10^{-4} mm. (light absorption: maxima, 3470, 3650, and 3840 A.; $E^{1\%}_{1\,cm.}$, 1300, 1560, 1250, respectively). The product was again adsorbed on a column of alumina, and the main band eluted, giving the *hydrocarbon* which polymerised rapidly on standing at 0°. Redistillation resulted in no

further increase in intensity of light absorption (maxima, 3510, 3660, and 3840 A.; $E^{1\%}_{1\,cm.}$, 1740, 2050, and 1500; ε, 46,500, 55,000, and 40,000, respectively) (Isler *et al.*, *Helv. Chim. Acta*, 1947, **30**, 1911, give light absorption of anhydrovitamin A: maxima, 3520, 3700, and 3910 A., $E^{1\%}_{1\,cm.}$, 1900, 2910, and 2620, respectively) (Found: C, 88·8; H, 10·75. $C_{20}H_{28}$ requires C, 89·5; H, 10·5%).

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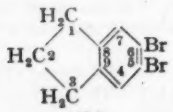
324. *A Contribution to the Study of the Mills–Nixon Effect. Dipole Moments of* 1 : 3-*Dioxaindane*, 1 : 4-*Dioxatetralin, and of Some of Their Nitro- and Bromo-derivatives.*

By H. D. SPRINGALL and G. C. HAMPSON, and (in part) C. G. MAY and H. SPEDDING.

A short summary of the work on the Mills–Nixon effect is given. The dipole moments of 1 : 3-dioxaindane and 1 : 4-dioxatetralin are interpreted in terms of the facility of electron release from the side rings to the aromatic nuclei. The observed differences are correlated with relative stereochemical stabilities of the excited ionic structures contributing to the molecules. The investigation is extended to the 5-nitro- and 5 : 6-dibromo-dioxaindanes and 6-nitro- and 6 : 7-dibromo-dioxatetralins. An analysis of the interaction moments is given which confirms the findings on the unsubstituted compounds.

MILLS and NIXON (*J.*, 1930, 2510) postulated, on stereochemical grounds, the fixation of the double bonds in the benzene nuclei of indane (hydrindene) (dicyclic, 8 : 9-bond single) and tetralin (dicyclic, 9 : 10-bond double), the stereochemical argument being much stronger in the case of indane. They adduced evidence for this fixation from observations of the chemical reactivity in coupling and bromination reactions of 5-substituted indanes and 6-substituted tetralins. This line of investigation was subsequently developed by Fieser and Lothrop (*J. Amer. Chem. Soc.*, 1936, **58**, 2050; 1937, **59**, 945) and by McLeish and Campbell (*J.*, 1937, 1103).

The work recorded below, on 1 : 3-dioxaindane and 1 : 4-dioxatetralin derivatives, begun in Oxford in 1933, was designed to amplify the investigation, started at the same time, of the Mills–Nixon effect by consideration of the dipole moments of 5 : 6-dibromoindane (I) and 6 : 7-dibromotetralin (II). As these two compounds proved somewhat inaccessible, the corresponding 5 : 6-dibromo-1 : 3-dioxaindane (III) and 6 : 7-dibromo-1 : 4-dioxatetralin (IV) were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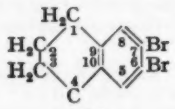
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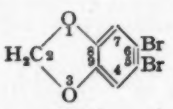
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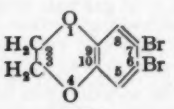

(IV.)

investigated. These compounds are formally analogous to the dibromo-indane and -tetralin and should show similar stereochemical side-ring effects; they have, moreover, the advantage of being much more readily accessible. The resultant dipole moments for these dioxa-compounds are, however, complicated by overlapping and opposed electromeric effects, and the interpretation of the results was deferred until the electrometrically less complex systems of the indane and tetralin series had been further investigated.

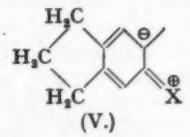
The preparation and dipole-moment investigation on the bromo-indane and -tetralin was completed in 1936 (Sidgwick and Springall, *J.*, 1936, 1532) and the dipole results interpreted provisionally as indicating probable bond fixation in indane (dicyclic, 8 : 9-bond single; component of moment due to Br·C·C·Br system = 1·78 D.; angle between bonds C_5–Br and C_6–Br, $\theta = 75° \pm 5°$), but free Kekulé resonance in tetralin (dicyclic, 9 : 10-bond aromatic; component of moment due to Br·C·C·Br system = 2·11 D.; angle between bonds C_6–Br and C_7–Br, $\phi = 60°$).

The work of Dolliver, Gresham, Kistiakowsky, and Vaughan (*J. Amer. Chem. Soc.*, 1937, **59**, 831) on the heats of hydrogenation of indane and various alkyl derivatives of benzene showed

that the benzene ring in indane is in fact stabilised by resonance by 42-kcals./mole, indicative of full Kekulé resonance and quite precluding bond fixation.

Sutton and Pauling (*Trans. Faraday Soc.*, **1935**, **31**, 939) had given a quantum-mechanical treatment to the Mills–Nixon effect problem, using the method of atomic orbitals. They showed that the chemical reactivity observations, which had been used as original evidence for bond fixation, could be explained in terms of activation energy changes, due to stereochemical strain effects stabilising, to a small extent, certain of the ionic excited structures, without invoking rigid fixation of aromatic nuclei in one or other of the Kekulé forms. Thus, the slight stabilisation, by the 5- and 6-membered rings respectively, of the excited structures (V) and (VI) is sufficient to account for the results of the coupling and bromination experiments.

These two lines of work rendered more urgent the application, which it had always been our intention to make, of a check on the interpretation of the dipole results as implying bond fixation in indane. This check was the measuring of the Br ⟷ Br distance in the dibromoindane and related compounds by means of electron diffraction. The Br ⟷ Br distance in the indane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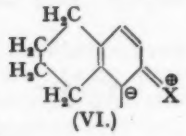
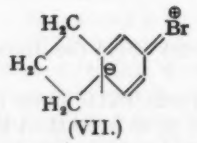

(VI.)

(X = NHAc or OH.)

derivative should be greater (calculated value 3·70 A.) if the 8 : 9-bond were indeed a fixed single bond ($\theta = 75°$) than the corresponding Br ⟷ Br distance (calculated value 3·39 A.) in *o*-dibromobenzene. When the electron-diffraction investigation was carried through (Kossiakoff and Springall, *J. Amer. Chem. Soc.*, **1941**, **63**, 2223; Springall, *Chem. and Ind.*, **1943**, **21**, 149) it was found that the Br ⟷ Br distances in the dibromo-indane, -tetralin, -*o*-xylene, and -benzene were identical (found value $3{\cdot}39 \pm 0{\cdot}02$ A.), thus confirming the free resonance in indane, to be expected from the work of Kistiakowsky and of Sutton and Pauling, and necessitating a reconsideration of the dipole-moment findings for the dibromo-indane and -tetralin in terms of $\theta = \phi = 60°$.

An interpretation of the low value of the Br·C·C·Br moment in the indane derivative in terms of changes in the stabilisation of ionic excited structures, following lines similar to those of Sutton and Pauling (*loc. cit.*), was offered by Kossiakoff and Springall, who suggested that the slight stabilisation by the Mills–Nixon strain of the side ring of structures such as (VII) and (VIII) could be responsible for the lowering of the Br·C·C·Br moment in 5 : 6-dibromoindane.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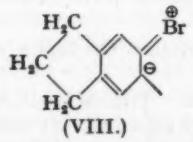
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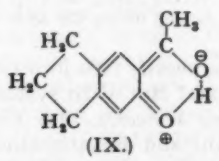
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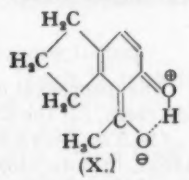
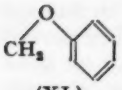

(X.)

Baker's observation (*J.*, **1937**, 477) of strong chelation in 5-hydroxy-6-acetylindane, and weak chelation in 5-hydroxy-4-acetylindane may be given a similar explanation in terms of the effect of the side ring on the stability of the ionic excited *o*-quinonoid structures (IX) and (X), the former being somewhat stabilised, and the latter somewhat destabilised.

In recent years the Mills–Nixon problem has been the subject of two theoretical investigations by the method of molecular orbitals. Wheland (*J. Amer. Chem. Soc.*, **1942**, **64**, 906) reconciled the reactivity of 5-substituted indanes with the virtual retention of Kekulé resonance in the aromatic ring, by consideration of the resonance integral of the dicyclic 8 : 9-bond. He envisages the possibility that the effect of the side ring is to stretch this bond. Longuet-Higgins and Coulson (*Trans. Faraday Soc.*, **1946**, **42**, 756) conclude that the steric effects of the side ring on the aromatic ring are to compress the bonds 7 : 8, 8 : 9, and 9 : 4 and to stretch the bonds 4 : 5, 5 : 6, and 6 : 7 of the aromatic ring. They develop from this point an alternative explanation of the activation energy effects responsible for the chemical reactivity observations, in terms of transition-complex stabilisation. This explanation has a formal resemblance to Kossiakoff and Springall's suggestion (*loc. cit.*) concerning the reconciliation of the dimensions and dipole moments of the dibromo-compounds, but Longuet-Higgins and Coulson are sceptical of the latter suggestion, though they do not advance an alternative.

Independently of work on the Mills–Nixon effect, the study of dipole moment results with the system (XI) has been considerably developed since 1933.



(XI.)

The work of Bergmann, Engel, and Sandor (*Z. physikal. Chem.*, 1930, *B*, **10**, 397), Bergmann and Tschudnowsky (*ibid.*, 1932, *B*, **17**, 107), Hampson and Sutton (*Proc. Roy. Soc.*, 1933, *A*, **140**, 562), Hampson, Sutton, and Farmer (*ibid.*, 1933, *A*, **143**, 147), Bennett (*Trans. Faraday Soc.*, 1934, **30**, 853), and Marsden and Sutton (*J.*, 1936, 599) on the dipole moments in the anisole series has led to the evaluation of (*a*) the interaction moments for various 4-substituted anisoles, (*b*) the angle θ_2 which the dipole moment of anisole (1·23 D.) makes with O–phenyl bond ($\theta_2 = 76°$).

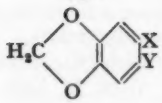
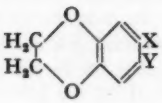
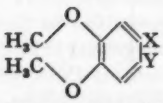
All this work provides a background against which it is possible to offer some explanation of the dipole-moment results obtained with the dioxa-indane and -tetralin derivatives.

The compounds examined were 1 : 3-dioxaindane and its 5-nitro- and 5 : 6-dibromo-derivatives, and 1 : 4-dioxatetralin and its 6-nitro- and 6 : 7-dibromo-derivatives.

The dipole-moment results for these compounds are given in Table I.

TABLE I.

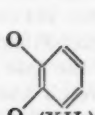
(Moments in D.)

	X = Y = H.	X = H, Y = NO_2.			X = Y = Br.		
Type.	μ.	μ (molecular).	NO_2 component.*	$\Delta\mu_{NO_2}$.*	μ (molecular).	Br·C·C·Br component.†	$\Delta\mu_{(Br)_2}$.†
	0·80	4·80	4·33	0·36	2·61	1·81	−0·31
	1·42	5·40	4·55	0·58	4·00	2·58	0·46
	1·37	5·18	4·34	0·37	3·75	2·38	0·26
Calculated model.							

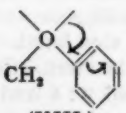
* Acting along the C–N bond.
† Acting along the axis of symmetry of the molecule.

The discussion of these results falls into two sections : the dipole moments of (1) the dioxa-side-rings, (2) the C·NO_2 and Br·C·C·Br systems.

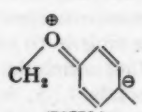
(1) *The Dioxa-side-ring Moments.*—(*a*) *Direction.* The molecular fragment (XII) must be planar, in both dioxaindane and dioxatetralin. The effect (XIII) will tend (i) to keep the CH_2 in the plane of the O–C_6H_5 and (ii) to increase the C–$\widehat{O}$–C angle above 110°. The operation of these two factors will cause the whole molecule of dioxaindane and probably that of dioxatetralin to be planar. (The molecular structure of these compounds will be determined by electron diffraction, to settle this point.) Even if the factors are not strong enough to hold the dioxatetralin rigidly planar, they will prevent any marked departures from that configuration. Symmetry considerations then necessitate that the resultant side-ring moments must act in



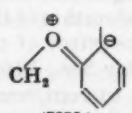
(XII.)



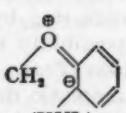
(XIII.)



(XIV.)



(XV.)



(XVI.)

the plane of the benzene nucleus and perpendicular to the dicyclic bond. From analogy with the anisole series it is almost certain that the sense of the side-ring moments must be with the negative ends towards the benzene ring. This is settled by the results for the mononitro- and dibromo-derivatives of the dioxa-compounds, which are compatible only with this assignment

of sense to the side-ring moments. This sense of the side-ring moments indicates that, as would be expected on general grounds, the ionic excited structures (XIV), (XV), and (XVI) contribute to the overall molecular structure.

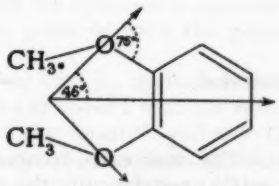
(*b*) *Magnitude.* It seems desirable to treat the dioxa-side-ring moments in terms of the moments for anisole and veratrole. The marked difference in magnitude between the moments of the 5- and the 6-membered side rings must come under consideration.

The specific planar configuration (XVII) of veratrole is taken as a model for the dioxa-ring compounds. The dipole-moment vector data for anisole enable the resultant moment of the configuration of veratrole (XVII) to be evaluated approximately by the expression

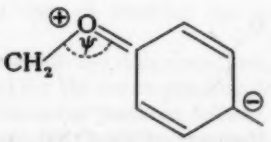
$$\mu_{\text{XVII}} = [2(1{\cdot}23 \cos 46°)] \times 0{\cdot}8 = 1{\cdot}37 \text{ D.}$$

(the factor 0·8 allows roughly for the " ortho-effect "—the mutual polarisation effect which depresses the resultant moment of two *o*-groups below the vector sum value; see, *e.g.*, Smallwood and Herzfeld, *J. Amer. Chem. Soc.*, 1930, **52**, 1919; Sidgwick and Springgall, *loc. cit.*).

The observed moment for 1 : 4-dioxatetralin, 1·42 D., is somewhat larger than the calculated " model " value. No great significance can be attached directly to the difference between 1·42 and 1·37 D. (especially in view of the approximations involved in the derivation of the calculated 1·37 D. value). Yet the difference may be real, for there is other evidence, from the nitrated and brominated compounds (see below), indicating that the electromeric or mesomeric release of electrons to the benzene nucleus from the oxygen atoms [by the participation of structures (XIV), (XV), and (XVI)] is more facile with the 6-membered dioxa-side-ring than with the methoxy-groups.



(XVII.)



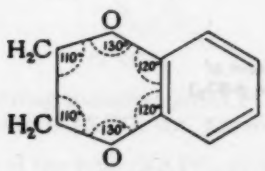
(XVIII.)

The observed moment for 1 : 3-dioxaindane, 0·80 D., is much lower than the calculated " model " value, indicating that electron release to the benzene nucleus is much less facile with the 5-membered dioxa-side ring than with the methoxy-groups.

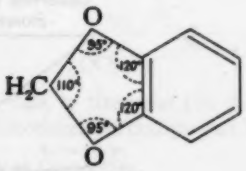
It is possible to suggest a treatment of these variations based on the following factors :

(i) The restriction, by ring closure, of free rotation about the $O–C_6H_5$ bonds in both the dioxa-side rings would help to stabilise the ionic excited structures (XIV), (XV), (XVI), etc., in these compounds relative to corresponding structures for the methoxy-compounds.

(ii) The ionic structures (XIV), (XV), and (XVI) involve the formation of a double bond in the side ring. The stereochemistry of the 6-membered ring favours this, while that of the 5-membered ring opposes it. Thus, in the ionic system (XVIII) the unstrained value for the angle ψ will be in the range 120—130°. This will be readily achieved in the 6-membered dioxa-side ring, but cannot be achieved in the 5-membered side ring. The angular dimensions shown



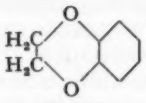
(XIX.)



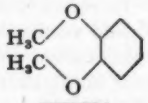
(XX.)

in (XIX) and (XX) have been derived then by assigning all angular strain to the $\widehat{COC}$ angle. (The work of Hampson and Sutton, *loc. cit.*, and Hampson, Sutton, and Farmer, *loc. cit.*, indicates that much less energy is required to change the O< angle than the CH_2< angle.) It is probable that the strain is to some extent shared by the C< angle, but (XIX) and (XX) show qualitatively

how the 6-membered side ring favours, and the 5-membered ring opposes, the large $\hat{COC}$ angle needed for the participation of structures (XIV), (XV), and (XVI).



(XXI.)



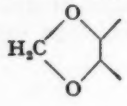
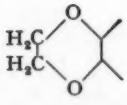
(XXII.)

(iii) In the 5-membered dioxa-side ring both the $CH_2 \rightarrow O$ bond moments have their positive poles on the same C atom. Mutual polarisation would tend to reduce these $CH_2 \langle^{O}_{O}$ bond moments relative to those of the systems (XXI) and (XXII). The effects of these factors are summarised in Table II.

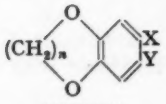
It seems not unreasonable to regard the observed high moment of the 6-membered ring as largely accounted for by factors (i) and (ii), and the low moment of the 5-membered ring as due to factors (ii) and (iii) preponderating over (i).

TABLE II.

Effect of factor on moment of side ring.

Side-ring type.	Factor (i).	Factor (ii).	Factor (iii).
	Increases	Decreases	Decreases
	Increases	Increases	—

(2) *The Moments of the* C·NO_2 *and* Br·C·C·Br *Systems.*—The mononitro-compounds of the dioxa-indane and -tetralin were investigated primarily to settle unequivocally the sense of the dioxa-side-ring moments; but the results are of some interest in themselves in that they give some light on the nature of the electromeric (mesomeric) conjugation interactions between the side ring and the groups X and Y in the general system (XXIII). In connection with these interactions the following are the starting points:

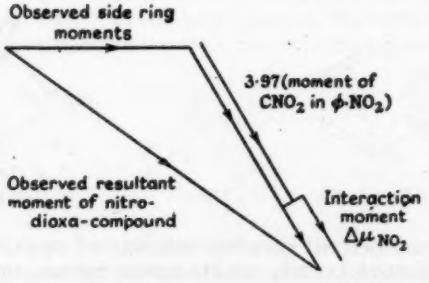


(XXIII.)

(*a*) From Kossiakoff and Springall's results (*loc. cit.*) on the dimensions of the indane and tetralin derivatives it is highly probable that in the dioxa-compounds (XXIII) the C–X and C–Y bonds make the normal aromatic angle of 120° with the adjacent aromatic bonds.

(*b*) Conjugated electromeric (mesomeric) interactions may occur between Y and the atom O *para* to Y, but not between Y and the O atom *meta* to Y.

From these considerations it is possible to construct the following simple vector model for the component moments of the nitro-compounds and to evaluate from this the mesomeric interaction moments, $\Delta\mu_{NO_2}$ operating along the C–N bond, between the dioxa-side ring and the C–NO_2 group.


Observed side ring moments
3·97(moment of CNO2 in ϕ·NO2)
Observed resultant moment of nitro-dioxa-compound
Interaction moment Δμ NO2

The resultant moments and the derived interaction moments for the (side-ring–NO_2) systems are given in Table I and are compared there with the corresponding data calculated for the

4-nitro-derivative of the specific configuration (XVII) of veratrole, using the known interaction moment 0·37 D. for *p*-nitroanisole (Marsden and Sutton, *loc. cit.*).

The values found for $\Delta\mu_{NO_2}$ indicate that ease of release of electrons to the aromatic system by the contribution of structure (XIV) is in the order 6-membered dioxa-ring > veratrole model > 5-membered dioxa-ring. This is the same order as was deduced from the moments of the unsubstituted compounds, though no individual significance can be attached to the difference between $\Delta\mu_{NO_2}$ for the veratrole model, 0·37 D., and for the 5-membered ring compound, 0·36 D.

These considerations enable some treatment to be given to the problem of the dipole moments of the Br·C·C·Br systems of 5 : 6-dibromo-1 : 3-dioxaindane and 6 : 7-dibromo-1 : 4-dioxatetralin (*i.e.*, the components of the resultant molecular moments due to the Br·C·C·Br systems).

In each of the compounds, the side-ring moment, the normal aromatic *o*-dibromo-moment, and any side-ring–Br interaction moment must, from symmetry considerations, all act along the same axis, that of the symmetry of the molecules, so that the required components are given by

$$\mu_{Br\cdot C\cdot C\cdot Br} = \mu_{molec.} - \mu_{side\ ring}$$

The values of these $\mu_{Br\cdot C\cdot C\cdot Br}$ are listed in Table I.

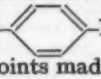
Each of these $\mu_{Br\cdot C\cdot C\cdot Br}$ terms may be regarded as made up of the normal aromatic *o*-dibromo-moment, the moment of *o*-dibromobenzene, 2·12 D. (Tiganik, *Z. physikal. Chem.*, 1931, *B*, **13**, 135), and the mesomeric interaction moment between the Br·C·C·Br system and the dioxa-side-ring, $\Delta\mu_{(Br)_2}$.

These interaction moments are given by

$$\Delta\mu_{(Br)_2} = \mu_{Br\cdot C\cdot C\cdot Br} - 2\cdot12$$

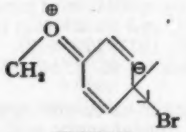
The values of the interaction moments are given in Table I.

From this point of view the problem is resolved into that of treating the variations in $\Delta\mu_{(Br)_2}$.

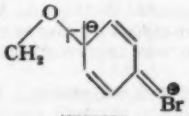
The values of $\Delta_{(Br)_2}$ derived from the observed moments of the dibromo-dioxa-compounds are compared in Table I with the reference value calculated for the corresponding 4 : 5-dibromo-derivative of the veratrole molecule (XVII), the calculation being made as follows. The work of Bennett (*loc. cit.*) and Marsden and Sutton (*loc. cit.*) has shown that the mesomeric interaction moment operating in *p*-bromoanisole has magnitude 0·15 D. acting along the O  Br axis, the negative end being towards the Br atom. This, taken with the general points made above in considering the $\Delta\mu_{NO_2}$ values, permits the calculation of the interaction moment $\Delta\mu_{(Br)_2}$ for 4 : 5-dibromoveratrole in the specific (XVII) configuration. In this case $\Delta\mu_{(Br)_2} = 2 \times 0\cdot15 \cos 30° = 0\cdot26$ D.

The " observed " value of $\Delta\mu_{(Br)_2}$ for the 6-membered side-ring compound, 0·46 D., is considerably higher than the model value, whereas for the 5-membered side-ring compound the value of $\Delta\mu_{(Br)_2}$, −0·31 D., is actually in the reverse sense to that of the model value.

In all these bromo-compounds the interaction moment is determined by a complex system of overlapping and opposed electromeric (mesomeric) effects, which can only be analysed qualitatively at present.



(XXIV.)



(XXV.)

(*a*) The strong inductive effect of the C⟶Br bond tends to increase the contribution of the ionic structures (XIV), etc., above the value for the unbrominated compound by promotion of structures of the type (XXIV), giving an interaction effect $\overset{+}{O}\longrightarrow Br$.

(*b*) The normal electromeric effect in the C_6H_5—Br system assisted by the inductive effect in the O⟵C bond tends to decrease contributions of the ionic structures (XIV), etc., below the value for the unbrominated compound by promotion of structures of the type (XXV), giving an interaction effect of the type $O\longleftarrow\overset{+}{Br}$

The work on *p*-bromoanisole and the rough calculations on the dibromoveratrole model show that, as would be expected, in the absence of steric complications due to side-ring formation, the $\overset{+}{O}\longrightarrow Br$ interaction predominates over the $\overset{+}{O}\longleftarrow Br$ interaction. The present results show that this $\overset{+}{O}\longrightarrow Br$ predominance is increased by the closure of the 6-membered ring, but that the resultant interaction effect is reversed ($\overset{+}{O}\longleftarrow Br$ predominating over $\overset{+}{O}\longrightarrow Br$) by the closure of the 5-membered ring.

The following possible explanation of these effects is offered. (*a*) The above consideration of the dipole results for the unsubstituted compounds and the mononitro-derivatives indicates that the ease of electron release from the oxygen atom to the benzene ring is in the order 6-membered dioxa-ring > veratrole model > 5-membered dioxa-ring [contribution of structures (XXIV) decreasing], so that from this point of view the $\overset{+}{O}\longrightarrow Br$ interaction effect should *decrease* in passing from dibromodioxatetralin through the dibromoveratrole model to the dibromodioxaindane. (*b*) The interpretation offered by Kossiakoff and Springall for the low moment of the Br·C·C·Br system in dibromoindane would lead to the view that the ease of electron release from the bromine atom to the benzene ring is in the order dibromodioxatetralin < dibromoveratrole < dibromodioxaindane [contribution of structures (XXV) increasing], so that the $\overset{+}{O}\longleftarrow Br$ interaction effect should *increase* in passing from the dibromodioxatetralin through the dibromoveratrole model to the dibromodioxaindane.

These two trends (*a*) and (*b*) would account for the observed variations in $\Delta\mu_{(Br)_2}$ interaction moment values in terms of reasonable variations in the contributions of ionic recited structures, due to steric effects of the Mills–Nixon type, but without any appeal to rigid bond fixation.

Experimental.

Preparation of Materials.—Benzene. Hopkin and Williams's thiophen-free benzene was frozen out four times, heated under reflux over phosphoric oxide, and distilled in a current of dry air into a receiver from which it could be pumped for immediate use.

1 : 3-*Dioxaindane.* Methylene sulphate was prepared by the action of fuming sulphuric acid on paraformaldehyde by the method of Delépine (*Compt. rend.*, 1899, **129**, 831; *Bull. Soc. chim.*, 1899, **21**, 1055) and Delaby (*ibid.*, 1926, **39**, 1612) as modified by Baker (*J.*, 1931, 1765). 1 : 3-Dioxaindane was prepared by the action of methylene sulphate on a mixture of catechol in acetone and sodium hydroxide in water (*idem*, *ibid.*, p. 1767). It distilled as a colourless oil, the fraction of b. p. 173°/756 mm. being used (Baker gives b. p. 173—174°/756 mm.).

1 : 4-*Dioxatetralin.* This was prepared by the action of ethylene dibromide on catechol, either (*a*) by using potassium hydroxide and water (Vorländer, *Annalen*, 1894, **280**, 205; Gattermann, *ibid.*, 1907, **357**, 373), or (*b*) by using potassium carbonate, copper bronze, and glycerol (Ghosh, *J.*, 1915, **107**, 1591). The compound distilled as a colourless oil at 212—216°/750 mm. The fraction of b. p. 99—100°/7 mm. was used (Gattermann gives b. p. 99—100°/7 mm.).

5-*Nitro*-1 : 3-*dioxaindane.* Piperonal was nitrated directly by nitric acid (*d* 1·42), and the required compound was separated from admixed nitropiperonal by the bisulphite reaction (Salway, *J.*, 1909, **95**, 1163). The product, recrystallised from alcohol, had m. p. 145° (Salway gives 145°; Jobst and Hesse, *Annalen*, 1879, **199**, 74, give 148°).

6-*Nitro*-1 : 4-*dioxatetralin.* 1 : 4-Dioxatetralin was nitrated by nitric acid (*d* 1·5), both in glacial acetic acid solution (Vorländer, *loc. cit.*). The product was recrystallized from alcohol; m. p. 122° (Vorländer gives m. p. 121°).

5 : 6-*Dibromo*-1 : 3-*dioxaindane.* Piperonylic acid was prepared by oxidation of piperonal by aqueous potassium permanganate (Fittig and Mielch, *Annalen*, 1869, **152**, 40), and its solution in aqueous sodium carbonate was brominated by means of bromine water (Robinson, *J.*, 1917, **111**, 913). The precipitated dibromo-compound was recrystallised from alcohol; colourless leaflets, m. p. 86° (Robinson, *loc. cit.*, gives m. p. 86°).

6 : 7-*Dibromo*-1 : 4-*dioxatetralin.* This was prepared by drawing the calculated quantity of bromine vapour, mixed with air, through a solution of 1 : 4-dioxatetralin in glacial acetic acid, at room temperature. The compound crystallised from the reaction mixture; it was collected, washed with water, and recrystallised from alcohol; m. p. 138° (Ghosh, *loc. cit.*, gives m. p. 138°).

Dipole-moment Determinations.—These determinations were made in benzene solution at 25° by the refractivity method, the heterodyne technique being used for the measurement of dielectric constant. Most of the measurements were carried out in Oxford (1934—1936), using the apparatus described by Sutton (*Proc. Roy. Soc.*, 1931, *A*, **133**, 668), Hampson, Farmer, and Sutton (*ibid.*, 1933, *A*, **143**, 147), Jenkins (*J.*, 1934, 481), and Jenkins and Sutton (*J.*, 1935, 609).

Some of the measurements on dioxa-indane and -tetralin were carried out in Manchester (1948), using a much more highly developed circuit for the measurement of dielectric constant. This circuit has been entirely based on a design prepared by Dr. L. E. Sutton and Dr. R. A. W. Hill in Oxford, and as the whole arrangement of the Oxford prototype will be discussed by them in a forthcoming publication, we only mention here the salient features. The measuring cell (as described by Jenkins and Sutton, *loc. cit.*) forms part of the variable oscillator circuit of a beat frequency oscillator ("B.F.O.") of which

the fixed oscillator operates at a frequency of 500 kc. The B.F.O. output is taken to the *X*-plates of a cathode-ray oscillograph ("C.R.O."), the *Y*-plates of which are fed from a fixed 1 kc. oscillator. The variable oscillator of the B.F.O. is tuned to 500 − 1 kc., the tuning being followed on the C.R.O. The measuring cell is then replaced, by means of a special low-capacity switch, in the variable oscillator of the B.F.O. by a standard Muirhead variable air condenser (Type A-411-F, 250 $\mu\mu$F.). The variable oscillator is then re-tuned to 500 − 1 kc., using the standard condenser alone. The capacity of the tuned standard condenser is then identical with that of the measuring cell. The equivalence of the two capacities is checked by the interchange of the measuring cell and the tuned standard condenser, no effect being produced on the C.R.O. figure. Special care is taken in the design of the H.T. power-packs, to ensure that the H.T. supply to the B.F.O. oscillators and the 1 kc. oscillator is completely stable to mains variations.

The results are given in the accompanying tables.

f_2.	d_4.	ε.	n^2.	P_2.	$_EP_2$.
		1:3-*Dioxaindane*.			
0·045212	0·8896	2·32040	2·26473	46·32	33·13
0·040521	0·8882	2·31570	2·26392	45·60	32·92
0·029828	0·8842	2·30415	2·26214	46·34	32·95
0·027885 *	0·88453	2·3060	2·2593	47·27	33·30
0·024002	0·8823	2·29848	2·26109	46·38	32·89
0·015731	0·8793	2·28900	2·25975	46·08	33·11
0·015234 *	0·88015	2·2910	2·2571	46·39	33·40
$_\infty P_T$ = 46·14 c.c.; P_E = 33·00 c.c.; P_O = 13·14 c.c.; whence μ = 0·80 D.					
		1:4-*Dioxatetralin*.			
0·049170	0·8916	2·41760	2·26732	79·62	37·28
0·038923	0·8889	2·39341	2·26647	79·13	37·66
0·030757	0·8860	2·37005	2·26439	79·97	37·38
0·029209 *	0·88680	2·366	2·2627	78·23	36·63
0·025326	0·8840	2·35228	2·26303	79·64	37·23
0·019682	0·8818	2·33496	2·26179	77·88	37·15
0·016754	0·8807	2·32610	2·26117	80·16	37·06
0·015337 *	0·88112	2·324	2·2594	78·70	36·35
$_\infty P_T$ = 79·40 c.c.; P_E = 37·29 c.c.; P_O = 42·11; whence μ = 1·42 D.					
		5-*Nitro*-1:3-*dioxaindane*.			
0·014598	0·8830	2·75825	2·26245	482·56	42·77
0·011286	0·8808	2·64731	2·26102	487·90	42·66
0·008203	0·8787	2·54561	2·26008	499·41	43·73
0·005425	0·8771	2·46001	2·25905	524·86	43·19
$_\infty P_T$ = 521·00 c.c.; P_E = 43·09 c.c.; P_O = 477·91 c.c.; whence μ = 4·80 D.					
		6-*Nitro*-1:4-*dioxatetralin*.			
0·013932	0·8830	2·89408	2·26392	617·28	48·21
0·012049	0·8816	2·80219	2·26297	619·67	48·67
0·008198	0·8792	2·63524	2·26119	642·46	48·43
0·005860	0·8776	2·51923	2·26015	627·32	48·96
0·003814	0·8762	2·4381	2·25920	656·64	49·91
$_\infty P_T$ = 652·5 c.c.; P_E = 48·84 c.c.; P_O = 603·66 c.c.; whence μ = 5·40 D.					
		5:6-*Dibromo*-1:3-*dioxaindane*.			
0·029396	0·9264	2·5659	2·76702	179·00	44·78
0·021774	0·9128	2·4894	2·76441	180·78	44·85
0·020435	0·9103	2·47512	2·26400	180·48	44·90
0·016194	0·9026	2·43252	2·26255	181·66	45·48
0·015857	0·9020	2·43004	2·26242	182·45	45·43
0·009491	0·8905	2·36751	2·26031	185·54	46·01
$_\infty P_T$ = 186·50 c.c.; P_E = 45·24 c.c.; P_O = 141·26 c.c.; whence μ = 2·61 D.					
		6:7-*Dibromo*-1:4-*dioxatetralin*.			
0·021805	0·9137	2·7237	2·26622	322·48	49·62
0·013933	0·8994	2·5616	2·26300	331·44	49·73
0·013390	0·8983	2·55008	2·26272	332·00	49·85
0·010562	0·8929	2·4912	2·26155	335·69	50·61
0·008435	0·8894	2·44223	2·26065	330·37	49·42
0·007653	0·8877	2·42734	2·26030	332·22	50·33
$_\infty P_T$ = 332·34 c.c.; P_E = 49·59 c.c.; P_O = 282·75 c.c.; whence μ = 4·00 D.					

* Results obtained in Manchester (1948).

We wish to acknowledge our deep indebtedness to Drs. Sutton and Hill for making their circuit design available to us, and for discussions; to Mr. K. A. Nelson who built the apparatus, in the work-

shops of the Chemistry Department in the University of Manchester, and who introduced certain modifications to allow the incorporation of available components; and to Dr. R. Feinberg, of the Department of Electrical Engineering of the University of Manchester, for discussions.

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We are also grateful to Imperial Chemical Industries and the Department of Scientific Industrial Research for grants, one of us (H. D. S.) to Lincoln College, Oxford, for a scholarship and to Magdalen College, Oxford, for a senior demyship, and two of us (H. D. S. and G. C. H.) to the Commonwealth Fund for Fellowships.

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THE UNIVERSITY OF MANCHESTER. [*Received, December* 31*st*, 1948.]

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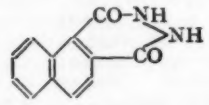
325. *Chemiluminescent Organic Compounds. Part IX. 5-Amino-ββ-naphthalaz-1 : 4-dione.*

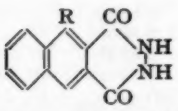
By BRIAN E. CROSS and H. D. K. DREW.

5-Amino-ββ-naphthalaz-1 : 4-dione has been synthesised from 3-hydroxy-2-naphthoic acid, and its chemiluminescence found to be weaker than that of 5-aminophthalaz-1 : 4-dione (luminol). Replacements taking place during diazotisation of 4-nitro-3-amino-2-naphthoic acid and the effect of Sandmeyer reagents on its diazonium salts under various conditions led to the formation of several new derivatives of 2-naphthoic acid.

IT was shown by Garwood and one of us (*J.*, 1939, 836) that αβ-naphthalaz-1 : 4-dione (I) and ββ-naphthalaz-1 : 4-dione (II; R = H) emitted a more intense chemiluminescence than did phthalaz-1 : 4-dione when treated with hydrogen peroxide under the conditions of Drew and Pearman (*J.*, 1937, 586).

The preparation of *5-amino-ββ-naphthalaz-1 : 4-dione* (II; R = NH_2) was undertaken in order that its chemiluminescence might be compared with that of luminol, and thus to determine whether the addition of a second benzene ring enhanced the chemiluminescence of 5-substituted phthalaz-1 : 4-diones.

(I.) 

 (II.)

When 3-acetamido-2-naphthoic acid, prepared from 3-hydroxy-2-naphthoic acid by amination and acetylation (B.P. 330,941; Allen and Bell, *Org. Synth.*, Vol. 22, p. 19), was nitrated in glacial acetic acid solution, it gave a mononitro-compound, which after removal of the acetyl group and reduction with zinc dust and acetic acid, readily condensed with phenanthraquinone to give a *benzphenanthrazinecarboxylic acid*; hence the nitro-group must be in the 4-position. This orientation was confirmed by acidifying a solution of the *4-nitro-3-amino-2-naphthoic acid* in hot dilute sodium hydroxide, to yield 4-nitro-3-hydroxy-2-naphthoic acid.

The preparation of 4-nitro-3-cyano-2-naphthoic acid from the foregoing amine proved troublesome owing to the great readiness with which the nitro-group was replaced during diazotisation and the Sandmeyer reaction. The required nitrile was however obtained in the form of its copper and nickel complexes by diazotising the amine by the method of Hodgson and Walker (*J.*, 1933, 1620), and immediately adding the diazo-suspension obtained to a solution of potassium cuprocyanide or nickelocyanide (Korczynski and Fandrich, *Compt. rend.*, 1926, **183**, 421) in a sufficiently large excess of potassium cyanide to neutralise all the sulphuric acid. Attempts to obtain the nitrile free from metal failed. The complexes could be decomposed only by boiling strong nitric acid, which not only removed the metal but also hydrolysed the cyano-group giving *4-nitronaphthalene-2 : 3-dicarboxylic acid*. The dicarboxylic acid was converted into its *anhydride* and thence into *1-nitronaphthalene-2 : 3-dicarboxyimide*. The imide was reduced with hydrogen and Adams's catalyst to the *1-amino-imide*, which on treatment with hydrazine yielded the required 5-amino-ββ-naphthalaz-1 : 4-dione (II; R = NH_2). When this compound was treated with hydrogen peroxide under the conditions of Drew and Pearman (*loc. cit.*), it emitted a fairly strong luminescence which was at first deep blue, but rapidly became almost white. The luminescence was considerably weaker than that of luminol under similar conditions. Other substituted ββ-naphthalaz-1 : 4-diones may be found to be more weakly chemiluminescent than the corresponding substituted phthalaz-1 : 4-diones.

The reaction of the diazo-suspension (Hodgson and Walker, *loc. cit.*) of 4-nitro-3-amino-2-naphthoic acid with potassium cuprocyanide, or potassium cyanide and copper bronze, in the presence of free sulphuric acid yielded a product, m. p. 270° (decomp.), which on decarboxylation gave α-nitronaphthalene and was undoubtedly *4-nitro-2-naphthoic acid*. Addition of the diazo-suspension (Hodgson and Walker, *loc. cit.*) to potassium nickelocyanide, prepared from nickel chloride (Korczynski and Fandrich, *loc. cit.*), or to aqueous nickel chloride, in both cases in the presence of free sulphuric acid, resulted in the replacement of the nitro-group by chlorine and the elimination of the amino-group, giving a chloro-2-naphthoic acid, m. p. 241°. A mixture of this acid and 3-chloro-2-naphthoic acid (Strohbach, *Ber.*, 1901, **34**, 4160; m. p. 216·5°) melted at *ca.* 190°. Hence the chloro-acid must be the previously unreported *4-chloro-2-naphthoic acid*. It is assumed that the elimination of the diazo-group during the formation of 4-nitro- and 4-chloro-2-naphthoic acids is effected by the reducing action of the cuprous (*cf.* Hodgson *et al.*, *J.*, 1941, 772; 1942, 744; 1947, 173) or nickel ions taking precedence over their more usual catalytic behaviour.

The nitro-group was not attacked during the reaction of the diazo-suspension with cuprous chloride and concentrated hydrochloric acid, which yielded *3-chloro-4-nitro-2-naphthoic acid*. However, when the amine was diazotised in a mixture of glacial acetic acid and concentrated hydrochloric acid both the nitro- and the amino-group were replaced by chlorine, giving *3 : 4-dichloro-2-naphthoic acid*.

Replacement of the nitro-group by hydroxyl readily took place during diazotisation in dilute sulphuric acid with the precipitation of *naphthalene-3-diazo-4-oxide-2-carboxylic acid*, a stable yellow compound, which was also formed by diazotisation using the conditions described by Hodgson and Mahadevan (*J.*, 1947, 325) for the preparation of solid diazonium sulphates from *o*-nitro-amines which readily form diazo-oxides under other conditions.

Experimental.

(M. p.s are uncorrected.)

3-Amino-2-naphthoic acid, prepared from the hydroxy-acid by fusion with zinc oxide and ammonium chloride (B.P. 330, 941) or by the method of Allen and Bell (*loc. cit.*), was acetylated giving 3-acetamido-2-naphthoic acid, m. p. 241—243°.

4-Nitro-3-acetamido-2-naphthoic Acid.—Concentrated nitric acid (5·2 g., 0·055 mole) in glacial acetic acid (10 c.c.) was stirred into a solution of 3-acetamido-2-naphthoic acid (11·45 g., 0·05 mole) in glacial acetic acid (90 c.c.), acetic anhydride (8 c.c.), and concentrated sulphuric acid (1·5 c.c.) at 65—70°. The mixture was cooled and allowed to stand overnight. The orange coloured product was collected and crystallised from aqueous ethanol, giving the *4-nitro-acid* as orange-yellow needles (11·2 g., 82%), m. p. 213° (decomp.) (Found : C, 57·2; H, 4·0; N, 10·55. $C_{12}H_{10}O_5N_2$ requires C, 56·95; H, 3·7; N, 10·2%).

4-Nitro-3-amino-2-naphthoic Acid.—The above acetyl derivative (19·8 g.) was hydrolysed with 15% methanolic potash (550 c.c.). The *amino-acid* crystallised from dilute acetic acid (charcoal) and then dilute ethanol in small orange-brown needles (12·5 g.), m. p. 240° (decomp.) (Found : C, 57·15; H, 3·8; N, 12·45. $C_{11}H_8O_4N_2$ requires C, 56·9; H, 3·45; N, 12·05%). It was insoluble in concentrated hydrochloric acid and in cold dilute sodium hydroxide solution, but dissolved in the latter on heating to form a deep-red solution which on acidification gave a precipitate of 4-nitro-3-hydroxy-2-naphthoic acid, m. p. and mixed m. p. with an authentic specimen, 239—240° (decomp.). *4-Nitro-3-benzamido-2-naphthoic acid* formed yellow needles from acetic acid and, after sublimation *in vacuo*, had m. p. 267° (Found : N, 8·65. $C_{18}H_{12}O_5N_2$ requires N, 8·35%). The *phenanthrazine* derivative of 3 : 4-diamo-2-naphthoic acid prepared by reduction of 4-nitro-3-amino-2-naphthoic acid in acetic acid solution with zinc dust, followed by the addition of phenanthraquinone, separated in small yellow crystals, m. p. 321—322° (decomp.), from a large volume of acetic acid (Found : N, 7·5. $C_{25}H_{14}O_2N_2$ requires N, 7·5%).

Reactions of 4-Nitro-3-diazo-2-naphthoic Acid Sulphate.—4-Nitro-3-amino-2-naphthoic acid was diazotised by the method of Hodgson and Walker (*loc. cit.*). A solution of the nitro-amine (0·58 g., 0·0025 mole) in boiling glacial acetic acid (10—12 c.c.) was rapidly cooled to 15°, and the fine suspension thus obtained was stirred into a solution of sodium nitrite (0·22 g., 0·003 mole) in concentrated sulphuric acid (2·7 c.c.) and glacial acetic acid (2—3 c.c.) at < 15°. Towards the end of the diazotisation varying amounts of a yellow solid separated. This diazo-suspension was used immediately in the following replacement reactions.

(*a*) The diazo-suspension was added with stirring to a solution of copper sulphate (0·66 g.) and potassium cyanide (0·75 g.) in water (5 c.c.) at 10—20°. After 1 hour the mixture was heated on a water-bath until effervescence ceased. Dilution with water precipitated a brown solid which, after crystallisation from aqueous ethanol (charcoal) and then acetic acid (90%), formed pale yellow needles of *4-nitro-2-naphthoic acid* (0·27 g.), m. p. 270° (decomp.) (Found : C, 60·8; H, 3·6; N, 6·4. $C_{11}H_7O_4N$ requires C, 60·85; H, 3·25; N, 6·45%). Decarboxylation of this nitro-acid in quinoline in the presence of copper bronze yielded α-nitronaphthalene, m. p. and mixed m. p. 58—59°.

(*b*) Addition of copper bronze (0·6 g.) to the mixture obtained by adding the diazo-suspension to a solution of potassium cyanide (1·0 g.) in water (5 c.c.) at 10—20° also yielded 4-nitro-2-naphthoic acid. The mixture was heated on a water-bath to complete the reaction. The brown solid which separated was worked up as in (*a*) giving pale yellow needles, m. p. and mixed m. p. with a specimen obtained by method (*a*), 270° (decomp.).

(*c*) The diazo-suspension was added with stirring to a solution of nickel chloride (0·6 g.) and potassium cyanide (0·9 g.) in water (5 c.c.) (Korczynski and Fandrich, *loc. cit.*) at 5—10°. The reaction was completed on the water-bath. When the mixture cooled, impure *4-chloro-2-naphthoic acid* separated as a reddish-brown solid which was crystallised from ethanol (charcoal), then dilute acetic acid, and finally benzene in slender cream-coloured needles (0·17 g.), m. p. 241° (mixed m. p. with a specimen of 3-chloro-2-naphthoic acid, 189—193°) (Found: C, 63·9, 64·2; H, 3·4, 3·35; Cl, 17·3. $C_{11}H_7O_2Cl$ requires C, 63·95; H, 3·4; Cl, 17·15%). Addition of the diazo-suspension to a solution of nickel chloride (3·0 g.) in water (20 c.c.) at 5—10°, followed by heating on the water-bath, also yielded crude 4-chloro-2-naphthoic acid which was worked up similarly; m. p. and mixed m. p. with a specimen prepared as above, 241°.

(*d*) *3-Chloro-4-nitro-2-naphthoic acid* was prepared by adding the diazo-suspension to a solution of cuprous chloride (2·0 g.) in concentrated hydrochloric acid (10 c.c.) at 10—15°. After being kept overnight the mixture was poured into water (50 c.c.). The yellow precipitate was crystallised from aqueous ethanol (charcoal) and then acetic acid; glittering yellow needles, m. p. 239—240° (decomp.) (Found: N, 5·8 Cl, 14·25. $C_{11}H_6O_4NCl$ requires N, 5·55; Cl, 14·1%).

(*e*) *Naphthalene-3-diazo-4-oxide-2-carboxylic acid.* (i) Ether (30 c.c.) was slowly added to the thick yellow suspension produced by keeping the diazo-suspension for 20 minutes. The yellow solid was collected and washed with glacial acetic acid–ether and then with ether. It had m. p. 130° (decomp.). Purification as in (ii) gave the *diazo-oxide* as dark yellow needles, m. p. and mixed m. p. with a specimen prepared by method (ii), 186° (decomp.). (ii) The diazo-oxide was also formed when the suspension produced by pouring a solution of 4-nitro-3-amino-2-naphthoic acid (0·58 g.) in concentrated sulphuric acid (*d* 1·84; 5 c.c.) on ice (15 g.) was diazotised with sodium nitrite (0·2 g.) in water (2 c.c.) at 5—10°. The amine dissolved to give a dark brown solution which immediately began to deposit a yellow solid. After 1—2 hours the solid was collected. It had m. p. *ca.* 165° (decomp.) and crystallised from aqueous ethanol in orange-brown needles (0·40 g.), m. p. 183—186° (decomp.). Crystallisation from ethyl acetate gave the pure diazo-oxide as dark yellow needles, m. p. 186° (decomp.) (Found: C, 61·7, 61·9; H, 3·1, 3·3; N, 13·4. $C_{11}H_6O_3N_2$ requires C, 61·7; H, 2·8; N, 13·1%).

(*f*) *4-Nitronaphthalene-2 : 3-dicarboxylic acid.* (i) The diazo-suspension (prepared from 2·32 g. of the amine) was rapidly stirred into a solution of copper sulphate (5·0 g.) and potassium cyanide (24·0 g.) in water (60 c.c.), the temperature being kept below 15° by the addition of ice. After 30 minutes the mixture was heated on a water-bath for 1 hour. When cold, the buff-coloured copper complex was collected and heated under reflux with 56% nitric acid (50 c.c.) for 45 minutes. The complex dissolved to give a green solution from which yellow needles separated on cooling. These needles still contained a little copper which was removed by heating under reflux with 56% nitric acid (30 c.c.) for 10 minutes. Crystallisation from water (charcoal) yielded *4-nitronaphthalene-2 : 3-dicarboxylic acid* which melted at 210° with gas evolution, solidified, and remelted at 229—230° (gas evolution). It recrystallised from water in long colourless blades, m. p. 233—234° (gas evolution); yield, 0·96 g. (37%) (Found: C, 54·95; H, 2·7; N, 5·7. $C_{12}H_7O_6N$ requires C, 55·2; H, 2·7; N, 5·35%). The *anhydride*, prepared by heating the acid under reflux with excess of acetic anhydride, had m. p. 237—238° (gas evolution) (Found: C, 58·8; H, 2·3. $C_{12}H_5O_5N$ requires C, 59·25; H, 2·05%). (ii) The preparation (using 1·16 g. of the amine) was carried out as in (i), except that an aqueous solution of nickel sulphate (2·8 g.) and potassium cyanide (12·0 g.) was used in place of the cuprocyanide solution. The buff-coloured nickel complex was decomposed as in (i). Crystallisation gave the dicarboxylic acid, m. p. and mixed m. p. with a specimen prepared by method (i), 231—232° (gas evolution).

3 : 4-Dichloro-2-naphthoic Acid.—Finely powdered sodium nitrite (0·2 g.) was added with stirring to a suspension of 4-nitro-3-amino-2-naphthoic acid (0·58 g.) in glacial acetic acid (12 c.c.) and concentrated hydrochloric acid (6 c.c.) at 10°. After a few minutes the solution was added to an aqueous solution of copper sulphate (0·66 g.) and potassium cyanide (0·75 g.) at 10—15°. The mixture was heated on the water-bath and cooled, and the product collected. Crystallisation from aqueous ethanol (charcoal), glacial acetic acid, and finally benzene yielded *3 : 4-dichloro-2-naphthoic acid* in slender cream-coloured needles, m. p. 233·5° (decomp.) (Found: C, 55·1; H, 2·8; Cl, 29·5. $C_{11}H_6O_2Cl_2$ requires C, 54·8; H, 2·5; Cl, 29·4%).

1-Nitronaphthalene-2 : 3-dicarboxyimide.—(*a*) An intimate, finely powdered mixture of the above anhydride (1·9 g.) and urea (0·6 g.) was heated with stirring to 180° and allowed to cool. The *imide* crystallised from absolute ethanol (charcoal) and glacial acetic acid in pale yellow rhombs (1·25 g., 66%), m. p. 270—271° (gas evolution) (Found: C, 59·3; H, 2·5. $C_{12}H_6O_4N_2$ requires C, 59·5; H, 2·5%). (*b*) An intimate, finely powdered mixture of 4-nitronaphthalene-2 : 3-dicarboxylic acid (0·85 g.) and ammonium carbonate (0·85 g.) was fused at 270—280° for 10 minutes. The brown product was worked up as in (i), giving small rhombs (0·4 g.), m. p. 267—268° which did not depress a specimen made by method (*a*).

1-Aminonaphthalene-2 : 3-dicarboxyimide.—A solution of the foregoing nitro-imide (0·8 g.) in ethyl acetate (100 c.c.) was shaken with Adams's catalyst (40 mg.) in hydrogen for 2 hours. A further quantity of catalyst (20 mg.) was added and the shaking continued for 1 hour. The solvent was removed, the bright-yellow residue taken up in glacial acetic acid and filtered, and the filtrate treated with charcoal and concentrated. The orange-yellow needles, m. p. 299—302° (decomp.), which separated were crystallised from absolute ethanol giving the pure *amine* in felted yellow needles, m. p. 302—303° (decomp.) (Found: C, 67·4; H, 3·4. $C_{12}H_8O_4N_2$ requires C, 67·9; H, 3·8%). Solutions of the amine were greenish-yellow in colour and had a strong greenish-blue fluorescence.

5-Amino-ββ-naphthalaz-1 : 4-dione.—The foregoing imide (0·2 g.) was heated under reflux with hydrazine hydrate (90%; 1·5 c.c.) and water (5 c.c.). The imide dissolved to a yellow solution which was filtered and acidified. The almost white precipitate of the *dione* was collected, dissolved in warm dilute ammonia, treated with charcoal, and filtered, and the filtrate acidified. Reprecipitation from dilute ammonia yielded the pure dione as a cream-coloured powder, m. p. 342—344° (decomp.) (Found: C, 60·6; H, 4·6. $C_{12}H_9O_3N_3,\frac{1}{2}H_2O$ requires C, 61·0; H, 4·25%). The dione was practically insoluble in organic solvents; it formed a sodium salt soluble in hot water but sparingly soluble in cold water.

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326. *A New Galactoside of Luteolin.*

By G. R. CLEMO and D. G. I. FELTON.

Chaerophyllum sylvestre has yielded a new glycoside, identified as a galactoside of luteolin.

DURING an investigation of possible alkaloidal constituents of hedge-row plants, the flowers and seed-pods of *Chaerophyllum sylvestre* (sheep's parsley) were studied. No basic crystalline material was found, but from the extracts there was isolated a yellow crystalline *compound*, m. p. 260° (decomp.), which appeared to be a glycoside and which was named chaerophyllin.

Extraction of the flowers and seed pods with dilute acetic acid, followed by basification, yielded an amorphous solid, whence continuous extraction with ethanol yielded a pale yellow crystalline substance (0·1—0·3% of the plant weight). Since the alkaloid content had been the original aim of the investigation, attention had been focussed on the plant towards the end of its flowering season. The material obtained in later stages of this investigation (from seed capsules only) proved to be unstable if kept, possibly owing to enzymes present in the plant at this time.

Chaerophyllin, after extraction, was insoluble in dilute acids and soluble in alkalis, yielding a deep yellow solution. It gave a greenish-brown ferric coloration. Its analysis was complicated by the presence of ethanol and water of crystallisation, but these were removed by prolonged heating in a vacuum at 160° and the empirical formula $C_{21}H_{20}O_{12}$ was obtained.

Hydrolysis with dilute hydrochloric or sulphuric acid afforded a yellow solid, m. p. 330° (decomp.), which, although insoluble in aqueous acids, dissolved in dilute sodium hydroxide yielding a deep yellow solution. Treatment of this solid with magnesium and alcoholic hydrogen chloride gave a deep pink coloration, discharged by alkali, a reaction indicative of a flavone, flavanol, or flavanone. An alcoholic solution gave a greenish ferric coloration suggesting a pyrocatechol structure. The filtrate from the hydrolysis was shown to contain galactose by comparison of the osazone with an authentic specimen.

The aglycone, which analysed as $C_{15}H_{10}O_6,H_2O$, formed a tetra-acetate, m. p. 226°. Hydrolytic fission with alcoholic potassium hydroxide gave an acidic and a ketonic fraction. The acidic fraction was obtained in amount too small to be identified, though it gave a green ferric coloration reminiscent of protocatechuic acid. The ketonic fraction gave a crimson 2 : 4-dinitrophenylhydrazone, which was identified as 3 : 4-*dihydroxyacetophenone* 2 : 4-*dinitrophenylhydrazone* by comparison with an authentic sample.

The m. p.s and analyses of the aglycone and its derivatives, its reactions, and its degradation product indicated that it was luteolin (5 : 7 : 3′ : 4′-tetrahydroxyflavone). The ultra-violet absorption spectrum, determined in ethanol, showed maxima at 2570 and 3530 A. (log ε_{molar} 4·21 and 4·30, respectively) and a minimum at 2860 A. (log ε_{molar} 3·93) in very good agreement with the values found for luteolin by Skarzynski (*Biochem. Z.*, 1939, **301**, 150) [max., 2580 (4·22) and 3550 (4·28); min., 2850 (3·86) A.].

An authentic specimen of luteolin was obtained through the courtesy of Professors F. Challenger and W. Bradley of Leeds, to whom are due our warmest thanks. Comparison, by the method of mixed m. p.s, of the aglycone with luteolin and of the corresponding tetra-acetates completely confirmed the identification. There was, however, insufficient material to attempt any determination of the position of attachment of the galactose moiety. The authors wish to draw attention to the incorrect values for the absorption spectrum of luteolin given by Shibata and Kimotsuki [*Acta Phytochim.* (*Japan*), 1922, **1**, 99].

EXPERIMENTAL.

Extraction.—The flowers and seed capsules of *Chaerophyllum sylvestre*, collected in June, were extracted (approximately 10 hours) with 4% acetic acid at 100°, and the solution filtered from the solid residue. After concentration in a vacuum to a small volume (100—150 ml.) and removal of deposited solid by centrifugation, the solution was basified with ammonium hydroxide (*d* 0·88), and the thick greenish-yellow precipitate was collected by centrifugation, washed several times with distilled water, pressed on a tile, and dried overnight (yield, 2—3%). The powdered, dry solid was then exhaustively extracted in a Soxhlet apparatus with ethanol. The residue on further extraction with acetone, chloro-

form, and light petroleum yielded no more material and was discarded. The deep-yellow ethanol solution was filtered and concentrated to a small volume. A yellowish and somewhat gummy precipitate separated from the cooled solution and was collected (yield from the extraction, 5—10%, *i.e.*, 0·1—0·3% of the plant material taken). Boiling the precipitate with 90% acetic acid gave a solution from which, on cooling, *chaerophyllin* separated as very pale yellow needles, m. p. 237° (decomp.). For analysis, it was recrystallised from aqueous ethanol (50%), whence it formed pale yellow, hair-like needles, m. p. 260° (decomp.) (Found: C, 50·9, 50·9, 51·3; H, 5·45, 6·2, 5·3. $C_{21}H_{20}O_{11}.C_2H_5OH,2\frac{1}{2}H_2O$ requires C, 51·2; H, 5·75. Found, on a sample dried in a high vacuum at 150°: loss, 13·1; C, 54·1; H, 5·1. $C_{21}H_{20}O_{11},H_2O$ requires loss, 13·5; C, 54·1; H, 4·7. Found, on a sample dried further in a vacuum at 160°; loss 4·5; C, 56·1; H, 5·1. $C_{21}H_{20}O_{11}$ requires loss, 3·9; C, 56·3; H, 4·5%).

Chaerophyllin was soluble in dilute sodium hydroxide, yielding a yellow solution, and gave a greenish-brown ferric coloration. It was insoluble in dilute, but dissolved in concentrated, acids.

Hydrolysis of Chaerophyllin.—Chaerophyllin (100 mg.) was boiled under reflux for 1 hour with 10% sulphuric acid (3 ml.); the suspension became a deeper yellow colour. After the solution had cooled, the precipitate was collected and washed with water, the filtrate and washings being reserved for the identification of the sugar residue, the presence of which was indicated by positive Fehling's and Molisch's reactions. The precipitate, on crystallisation from aqueous ethanol, formed small yellow needles, m. p. 330°, undepressed on admixture with an authentic sample of luteolin (Found: C, 59·1, 59·2; H, 3·8, 3·75. Calc. for $C_{15}H_{10}O_6,H_2O$: C, 59·3, H, 3·95%. Ultra-violet light absorption in ethanol: maxima at 2570 and 3530 A., log $\varepsilon_{max.}$ 4·21 and 4·30, respectively). The product was soluble in concentrated mineral acids and in dilute alkalis, but insoluble in water, dilute acids, and sodium carbonate solution. With alcoholic ferric chloride it gave a green colour, indicative of a phenolic compound of pyrocatechol type, and with magnesium and alcoholic hydrogen chloride a bright pink colour typical of a flavone.

Acetylation.—The aglycone (100 mg.) was heated at 105° for 15 minutes with acetic anhydride (1 ml.) and anhydrous sodium acetate (300 mg.). After cooling, the mixture was poured into an excess of water, triturated, and set aside overnight. The precipitate was then collected, washed, dried, and recrystallised from ethanol, forming long, silky needles, sintering at 210—212° and melting at 226° (Found, C, 60·8; H, 4·0. Calc. for $C_{15}H_6O_6(CO\cdot CH_3)_4$: C, 60·6; H, 4·0%). The mixed m. p. with tetra-acetyl-luteolin (prepared similarly from authentic luteolin) was identical.

Hydrolytic Fission.—The aglycone (100 mg.) was heated under reflux for 3 hours with alcoholic potassium hydroxide (4 ml.; 50%). The solution was acidified with sulphuric acid and then treated with excess of sodium carbonate. The filtered solution was continuously extracted with ether, the extract dried and evaporated, and the very small residue of oil taken up in ethanol and treated with an ethanolic solution of 2 : 4-dinitrophenylhydrazine sulphate. The small amount of red precipitate was recrystallised from a very small quantity of ethanol, m. p. 246—247° (decomp.), undepressed on admixture with an authentic sample of 3 : 4-dihydroxyacetophenone 2 : 4-dinitrophenylhydrazone (see below).

The residue from the continuous ether extraction was acidified and again extracted continuously with ether. After drying and evaporation, the residue was a very small amount of an oily solid, which gave a greenish ferric coloration and dissolved in sodium hydrogen carbonate with effervescence. Attempted sublimation was unsuccessful, and no crystalline product was isolated.

Identification of Galactose.—The aqueous filtrate from the hydrolysis of the glycoside was treated with excess of barium hydroxide solution and then with carbon dioxide to remove the barium. The filtered solution was concentrated in a vacuum to a small volume (approx. 1 ml.) and treated with an aqueous mixture of phenylhydrazine and acetic acid. On warming the mixture to 100°, a yellow precipitate began to separate after 9 minutes. After being heated for ½ hour, the mixture was cooled, and the precipitate collected, washed, and recrystallised from ethanol, yielding sheaves of yellow, hair-like needles, characteristic of galactosazone, m. p. 198°, and undepressed on comparison with an authentic sample.

3 : 4-*Dihydroxyacetophenone* 2 : 4-*dinitrophenylhydrazone.*—This *derivative* was prepared in the usual way from 3 : 4-dihydroxyacetophenone (Rosenmund and Lohfert, *Ber.*, 1928, **61**, 2601) and crystallised from ethyl acetate as crimson prisms, m. p. 247° (decomp.) (Found: C, 50·4; H, 3·5. $C_{14}H_{12}O_6N_4$ requires C, 50·6; H, 3·6%).

One of us (D. G. I. F.) wishes to thank the University of Durham for the award of an I.C.I. Fellowship.

UNIVERSITY OF DURHAM, KING'S COLLEGE,
NEWCASTLE-UPON-TYNE, 1.

[*Received, January 26th*, 1949.]

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327. *Oxidation of Some Simple Carbohydrates by Periodate in Phosphate and Other Buffers.*

By D. J. BELL, ANNE PALMER, and (in part) A. T. JOHNS.

Periodate oxidation of certain carbohydrates has been examined in the presence of phosphate and other buffers. At pH 7·5, phosphate tends to promote "over-utilisation" of periodate, and the reactions proceed more rapidly than in carbon dioxide–bicarbonate or maleate buffers. It is therefore suggested that a complex ion may be formed between phosphate and periodate, and that the mode of oxidation by this complex differs from that by periodate itself.

In phosphate buffer (pH 7·5) 2 : 3 : 4-trimethyl glucose yielded considerably less than the expected one mole of formaldehyde. The "missing" formaldehyde was accounted for as formic acid. Increase in periodate concentration relative to phosphate favoured formaldehyde production, but this never approached the theoretical value. On the other hand, oxidation in

phthalate (pH 6·2) was very slow, but finally produced one mole of formaldehyde and no formic acid. In contrast, the oxidation of 1 : 3 : 4-trimethyl fructose, in phosphate, took place "normally" to give one mole of formaldehyde.

3-Methyl glucose behaved "normally" in bicarbonate (7·5), but in phosphate (7·5) a low yield of formaldehyde resulted. 3 : 4 : 6-Trimethyl fructose, 3 : 4-dimethyl fructose, and fructose itself could not be made to yield the correct amounts of formaldehyde in a number of buffers and at different pH.

In phosphate, α-methylglucoside reduced considerably more than the theoretical two moles of periodate and the yield of formic acid tended to exceed somewhat the theoretical amount of one mole.

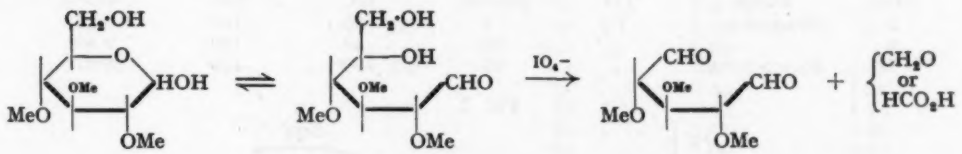
THIS paper extends observations made by one of us (Bell, *J.*, 1948, 992) when it was shown that the periodate ion, buffered to pH 7·5 by phosphate, reacted rapidly with certain, but not all, partly methylated sugars [each containing a ·CH(OH)·CH_2·OH group] examined, to yield the anticipated molar proportion of formaldehyde. The formaldehyde was determined and identified as the dimedon derivative. On the other hand, a number of the sugars reacted slowly, and in an "anomalous" manner in that the yield of formaldehyde was considerably less than that demanded by theory. The general procedure was, however, suitable for a small-scale qualitative test for the ·CH(OH)·CH_2·OH group in partly methylated sugars.

In order to facilitate certain researches we decided to investigate further several of these anomalous oxidations.

A notable example in the original work is provided by 2 : 3 : 4-trimethyl glucose. This substance exists only as a syrup and is known readily to part with the elements of water to form the 1 : 6-anhydro-derivative (Irvine and Oldham, *J.*, 1921, **119**, 1744). We therefore employed only material (prepared from crystalline 2 : 3 : 4-trimethyl 1 : 6-anhydroglucose) which had been freshly chromatographed on the silica–water partition column (Bell, *J.*, 1944, 473) and which, so far as could be ascertained, was analytically homogeneous.

We first sought to discover why the yield for formaldehyde from 2 : 3 : 4-trimethyl glucose was, in presence of phosphate, always below the theoretical, and also sought conditions under which the sugar could be induced to behave in the manner appropriate to its structure. We noted that previous workers had also failed to obtain theoretical yields of formaldehyde from 2 : 3 : 4-trimethyl glucose under a variety of conditions. Ariyama and Kitasato (*J. Biochem. Japan*, 1937, **25**, 357), using strongly acidified periodic acid, found only 0·02 mole of formaldehyde whilst Jeanloz (*Helv. Chim. Acta*, 1944, **27**, 1509) after 17-hours oxidation in carbon dioxide–bicarbonate buffer (containing sulphate) found 0·17 mole.

We confirmed our original observations that formaldehyde formation in phosphate buffer (pH 7·5) ceased long before the theoretical yield was attained. We examined the end-products of the reaction and found that a volatile acid having the properties of formic acid was produced in amounts which virtually accounted for the missing formaldehyde if it is assumed that only the primary alcohol group of the sugar was detached from the molecule :



Furthermore, the consumption of periodate in these experiments was considerably in excess of theoretical. Results comparable with those of the phosphate experiments were obtained using carbon dioxide–bicarbonate buffer at pH 7·5 [containing SO_4'' and prepared according to Jeanloz (*loc. cit.*)]. Price and Croll (*J. Amer. Chem. Soc.*, 1938, **60**, 2726) in the instance of pinacol, and both Reeves (*ibid.*, 1941, **63**, 1476) and Jeanloz in the instances of some carbohydrates, have emphasised the influence of pH on periodate oxidations of glycol groups. It seemed to us possible that two factors, at least, might operate in our anomalous oxidations. First, the effect of pH, and, secondly, an effect caused by complex-ion formation between periodate and phosphate in the one buffer, possibly between sulphate or carbonate in the other. If such a complex oxidant were in fact formed, its mode of attack on glycols might well pursue a course different from that of periodate alone. Thus, the effect of increasing the periodate concentration when phosphate was present was to increase the yield of formaldehyde with an exactly corresponding decrease in formic acid. On the other hand, oxidation in phthalate buffer (pH 6·2), where we did not expect complex formation, resulted in a slow liberation of formaldehyde, which,

however, ultimately reached 97% of theory after 650 hours. Here, the theoretical amount of periodate was reduced and no volatile acid formed.

Our results are summarised in Table I, and the progress of the reactions is represented graphically in Fig. 1.

FIG. 1.

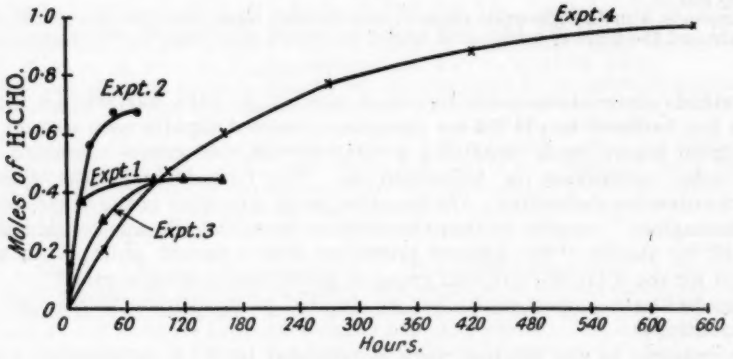


TABLE I.

Oxidation of 2 : 3 : 4-*trimethylglucose.*

Note.—In this and the following tables, molecular proportions are expressed in relation to 1 mole of substance oxidised. The columns " oxidation times " express, under " 1st " the time of the first measurement at which the reaction had reached a steady state, and under " 2nd " the time of the last measurement made, confirming completion of the oxidation. Results at " 1st " and " 2nd " times are identical.

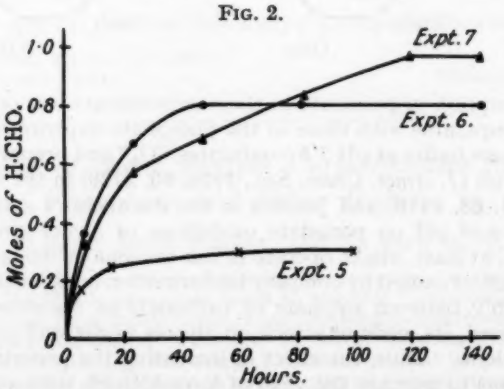
Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.). 1st.	2nd.	Found. CH_2O (moles).	$H{\cdot}CO_2H$ (moles).	IO_4^- (moles) reduced.
1	Phosphate	7·5	4·1	90	120	0·44	0·55	4·1
2	,,	,,	9·3	48	72	0·66	0·27	—
3	Bicarbonate	,,	4·3	110	160	0·44	0·45	4·3
4	Phthalate	6·2	2·6	650	—	0·97	0·00	1·0

TABLE II.

Oxidation of 3-*methyl glucose.*

Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.). 1st.	2nd.	Found CH_2O (moles).
5	Phosphate	7·5	4	60	100	0·30
6	,,	,,	33	48	150	0·80
7	Bicarbonate	,,	33	120	150	0·97

FIG. 2.

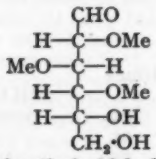


A second " anomalous " oxidation previously encountered was that of 3-methyl glucose. In acid periodate, Ariyama and Kitasato (*loc. cit.*) obtained 0·51 mole of formaldehyde. Bell

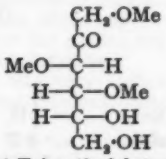
(*J.*, 1948, 992), using phosphate (pH 7·5) and four moles of IO_4^- per mole of sugar, found slow oxidation which ceased when about 0·25 mole of formaldehyde had been liberated. We have now found that, by use of a very large excess of periodate in bicarbonate buffer (pH 7·5) but not in phosphate, 0·97 mole of formaldehyde is formed. Results are summarised in Table II, and the courses of the reactions set forth in Fig. 2.

From experiments 5 and 6 it is again evident that pH is not the sole factor controlling formaldehyde formation. The suggestion of competitive oxidation indicated in experiments 1 and 2 is once more present.

A further point of interest is emphasised if the oxidation of 1 : 3 : 4-trimethyl fructose is compared with that of 2 : 3 : 4-trimethyl glucose. Theoretically, both sugars would be expected to behave in closely similar manner towards periodate since both have the same structure about carbons 3, 4, 5, and 6, when reacting in the open-chain form :



2 : 3 : 4-Trimethyl *aldehydo*-D-glucose.



1 : 3 : 4-Trimethyl *keto*-D-fructose.

1 : 3 : 4-Trimethyl fructose readily forms the theoretical one mole of formaldehyde in phosphate buffer, but, as in the case of 2 : 3 : 4-trimethyl glucose, over-consumption of periodate also takes place (Table III). This, however, is not so marked with the fructose compound. It is therefore possible, by formaldehyde determination, to detect and determine 1 : 3 : 4-trimethyl fructose in the presence of 1 : 3 : 4 : 6-tetramethyl fructose, since the latter does not react with periodate in phosphate unless contact with the reagents is prolonged beyond the time when formaldehyde liberation from the former is complete.

Experiments on the oxidation of 3 : 4 : 6-trimethyl fructose were also carried out. This sugar is known only as a syrup. Despite every care during the preparation of our material from trimethyl inulin, we are convinced that it is not completely homogeneous. Through the

TABLE III.

Oxidation of 1 : 3 : 4-*trimethyl fructose and* 1 : 3 : 4 : 6-*tetramethyl fructose in phosphate* (pH 7·5).

Serial No. of Expt.	Sugar.	Amount of IO_4^- (moles).	Oxidation times.	Found. CH_2O (moles).	Found. IO_4^- (moles) reduced.
8	Me_3	2·3	55	1·0	—
			70	1·0	—
9	,,	2·3	70	0·99	—
			120	0·99	—
10	,,	2·8	5	0·30	0·48
			24	0·80	1·28
			29	0·97	1·28
			48	1·01	1·50
			72	1·00	1·62
			96	1·00	1·81
11	Me_4	2·0	2	0	0
			22	0	0
			70	0·05	0·17
			94	0·09	0·30
			120	0·14	0·40

TABLE IV.

Oxidation of fructose (pH 7·5).

Serial No. of Expt.	Buffer.	Amount of IO_4^- (moles).	Oxidation times (hrs.). 1st.	2nd.	Found. CH_2O (moles).	Found. IO_4^- (moles) reduced.
12	Phosphate	6	3	96	1·68	4·6
13	,,	20	3	16	1·71	—
14	Maleate	6	24	48	1·74	4·1

TABLE V.

Oxidation of 3 : 4-*dimethyl fructose in phosphate* (pH 7·5).

Serial No. of Expt.	Amount of IO_4^- (moles).	Oxidation times (hrs.).	Found. CH_2O (moles).	Found. IO_4^- (moles) reduced.
15	4·4	29	1·72	1·78
		46	1·78	2·07
		70	1·78	2·21
		96	1·77	2·41

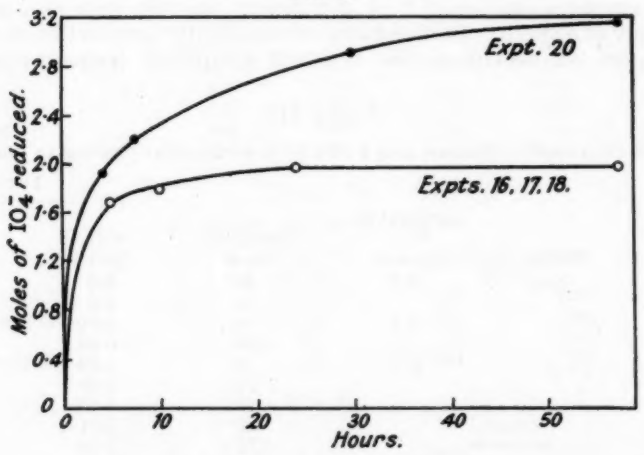
TABLE VI.

Oxidation of α-methylglucoside.

Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.). 1st.	2nd.	Found. HCO_2H (moles).	Found. IO_4^- (moles) reduced.
16	Phthalate	6·2	2·8	72	96	—	1·98
17	,,	,,	3·5	48	78	1·0	2·0
18	Bicarbonate	7·5	3·5	24	48	1·1	2·0
19	Phosphate	,,	2·8	24	—	—	2·8
20	,,	,,	3·5	48	96	1·3	3·2

kindness of Dr J. K. N. Jones of Bristol University, an independent paper chromatogram was carried out by the elegant technique of Hirst, Hough, and Jones (this vol., p. 928). By this means a fast-running minor component was detected, in addition to the trimethyl sugar.

FIG. 3.



Similar results were obtained in this laboratory. From this evidence and other facts, one of which is recorded below, we believe that a proportion of 3 : 4 : 6-trimethyl fructofuranose when kept in the dry state, and also in aqueous solution, can undergo some degree of autocondensation to form a non-reducing substance, probably a hexamethyl difructose anhydride (cf. Montgomery, *Adv. in Carb. Chem.*, 1946, **2**, 253). A freshly prepared sample of 3 : 4 : 6-trimethyl fructose yielded on periodate oxidation 0·9 mole of formaldehyde, but when the dry syrup or a solution in water was kept the formaldehyde yield fell to 0·80—0·82 moles on oxidation in phosphate. Oxidation at pH 7·5 in maleate or at 6·2 in phthalate gave similar results. There seemed to be a slight tendency to over-reduction of periodate. These findings are comparable with the results reported by other workers on the oxidation of fructose itself, *e.g.*, by Fleury and Lange (*Compt. rend.*, 1939, **209**, 219) using acid periodate, and both Reeves (*loc. cit.*) and Jeanloz (*loc. cit.*) using bicarbonate buffer at pH 7·5. Jeanloz also found that, although the yield of formaldehyde from fructose varied according to the pH, the theoretical value of 2 moles was never reached; results were, *e.g.*, at 7·5, 1·74 mole, at 8·8, 1·78 mole, and at 10·0, 1·22 mole. Our

own results (cf. Jackson, "Organic Reactions," 1944, **2**, 341) on fructose at pH 7·5 are shown in Table IV.

Oxidation proceeded more rapidly in phosphate than in maleate buffer, without significantly altering the final yields of formaldehyde. The amount of periodate consumed in both buffers is less than the five moles expected.

Similar results were also obtained when 3 : 4-dimethyl fructose was investigated in phosphate buffer (Table V).

In conclusion, we append data relating to the oxidation of α-methylglucoside (Table VI and Fig. 3). In these examples it will be seen that use of phosphate buffer leads to marked over-utilisation of periodate.

Experimental.

Conditions used for oxidation, determination of formaldehyde and consumption of periodate were identical with those described by Bell (*J.*, 1948, 992). Blank experiments on formaldehyde determinations were found to be essential since the acetate buffer tends to decompose when kept, to yield a substance giving a precipitate with dimedon. Molarity of the buffers used for the oxidations varied from 0·066 to 0·2; investigation showed that, in all experiments, buffering was adequate.

In certain experiments, where the concentration of periodate was very high and where K^+ was present, aliquots of the solutions for future examination were pipetted into individual flasks, before any separation of potassium salts could take place.

Determination of Formic Acid (" *Volatile Acid* ").—The majority of these determinations were carried out by one of the authors as "unknowns." An aliquot of the reaction mixture (usually equivalent to about 5 mg. of carbohydrate) was treated with 2 ml. of pure ethylene glycol in order to destroy excess of periodate. After the solution had been kept for one hour, its pH was adjusted (red to thymol-blue, pH <1·2) by addition of saturated potassium hydrogen sulphate solution. The volume was then made up to 15 ml. A suitable aliquot (1 or 2 ml.) was pipetted into the distillation apparatus (Markham, *Biochem. J.*, 1942, **36**, 790), and 50 ml. of distillate were collected. This was titrated in a stream of CO_2-free air with CO_2-free 0·01N-NaOH using phenol-red as indicator. Experimental error was established as ±0·05%. A blank determination was simultaneously carried out on the reagents; this was of the order of 0·02 ml. of 0·01N-NaOH. The foregoing procedure is similar to that of Pirie (*Biochem. J.*, 1946, **40**, 100). Confirmation that the volatile acid was in fact formic acid was obtained by redistillation of the first distillate after boiling under reflux with mercuric sulphate (Friedemann, *J. Biol. Chem.*, 1938, **123**, 161). In experiments 1 and 3 on 2 : 3 : 4-trimethyl glucose, concentration of the distillates at pH 10 yielded material giving the formhydroxamic acid spot reaction.

Two of the authors (A. P. and A. T. J.) gratefully acknowledge grants from the Agricultural Research Council.

Biochemical Laboratory, Cambridge. [*Received, November 24th*, 1948.]

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328. *Methyl-substituted Long-chain Acids. Part II.*

By S. David, N. Polgar, and Sir Robert Robinson.

In a previous communication (Polgar and Robinson, *J.*, 1945, 389), which is regarded as Part I of this series, it was shown that phthioic acid might be 2 : 12 : 18-trimethyltricosoic acid.* Since biological tests with the synthetic substance of this constitution indicated that, similarly to the natural acid, it produced lesions resembling those of tuberculosis, it became of interest to study the structural requirements for this effect.

The present paper reports syntheses of 2 : 12 : 18-*trimethyltricosa*-12 : 18-*dienic acid*, 2-*methyltricosoic acid*, and 2 : 12-*dimethyltricosoic acid*. It is found that 2 : 12 : 18-trimethyltricosa-12 : 18-dienoic acid also shows marked activity in producing these specific tissue reactions; 2 : 12-dimethyltricosoic acid, with the $C_{(18)}$-methyl of 2 : 12 : 18-trimethyltricosoic acid absent, is slightly active; whereas 2-methyltricosoic acid is inactive.

The preparation of 12 : 16-*dimethyltricosoic* and 12 : 15-*dimethyldocosoic acid* is also described.

Previous investigations (Polgar and Robinson, *loc. cit.*) indicated that phthioic acid is a methyl-substituted long-chain acid with three such branches, probably 2 : 12 : 18-trimethyltricosoic acid,* and this structure has been shown to be feasible by the synthesis of the substance.

* All the aliphatic acids mentioned in Part I (*J.*, 1945, 389) were numbered according to the Geneva system, $\overset{5}{C}\text{–}\overset{4}{C}\text{–}\overset{3}{C}\text{–}\overset{2}{C}\text{–}\overset{1}{CO_2H}$; undecanoic and higher acids were given the Geneva termination "anoic," but the "an" was omitted for decoic and lower acids. Nomenclature in the present and succeeding paper follows that more recently used in the Journal. The C of the CO_2H is not enumerated, numerals 1, 2, 3, etc. corresponding to α, β, γ, etc.; Greek letters are used with trivial names, numerals with names which embody a numerical root; alternatively, the hydrocarbon name terminated by a numeral and "carboxylic acid" may be used. Thus, *e.g.*, $CH_3{\cdot}CH_2{\cdot}CHBr{\cdot}CO_2H$ may be termed α-bromobutyric or 1-bromopropane-1-carboxylic acid, and $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CHBr{\cdot}CO_2H$ 1-bromohexoic or 1-bromopentane-1-carboxylic acid; the acid now termed 2 : 12 : 18-trimethyltricosoic acid was previously (*loc. cit.*) termed 3 : 13 : 19-trimethyltricosanoic acid.—Editor.

Further studies of the natural acid are in progress and have led to the conclusion that " phthioic acid " is not homogeneous. Hence our conclusions can only approximate to the truth. Meanwhile biological tests indicated that 2 : 12 : 18-trimethyltricosanoic acid exhibits activities similar to those obtained with the natural substance, whereas other methyl-substituted long-chain acids described in Part I, *e.g.*, 1 : 12-dimethylpentacosoic, 12 : 16 : 20-trimethyldocosoic, and 1 : 12 : 16 : 20-tetramethyldocosoic acid, were found to be inactive in this respect. This pointed to a high degree of structural specificity and it became necessary to study closely the effect of modifications in the structure of 2 : 12 : 18-trimethyltricosoic acid on the biological activity.

The introduction of ethylenic linkages, while otherwise retaining the structure of 2 : 12 : 18-trimethyltricosoic acid, seemed of interest in view of the possibility that dehydrogenation reactions may take place in the organism resulting in similar unsaturated intermediates. Esters of unsaturated acids with non-conjugated ethylenic linkages such as linoleic or linolenic acid are stated to be capable of producing certain tissue reactions owing to their transformation in the intercellular medium into insoluble semi-solid, amorphous substances which gradually acquire the property of retaining phenolised fuchsin with the same tenacity as do acid-fast bacilli (Hass, *Arch. Path.*, 1938, **26**, 956, 1183, 1196). Moreover, the caseation process occurring in tuberculous lesions has also been claimed earlier to be caused by the presence of unsaturated acids and it has been suggested that unsaturated fatty acids suppress the autolytic enzymes somewhat in proportion to their degree of unsaturation (Jobling and Petersen, *J. Exp. Med.*, 1914, **19**, 251, 459, and other papers). In this connexion a suggestion made by Robertson *et al.* (*J. Amer. Chem. Soc.*, 1944, **66**, 1894) is also of interest, namely, that unsaturated acids with non-conjugated ethylenic linkages are oxidation catalysts, oxidising, for example, sulphides to sulphoxides.

The synthesis of 2 : 12 : 18-*trimethyltricosa*-12 : 18-*dienoic* acid was, therefore, undertaken and it was hoped that biological examination of this acid and comparison of its action with that of the corresponding saturated acid would also throw some light upon the nature of this action.

Its synthesis was achieved in the same general way as that of the corresponding saturated acid (Part I, *loc. cit.*). The required intermediate, 12 : 18-*dimethyldocosa*-1 : 12 : 18-*triene* (I), was obtained by reaction of 6-methyldec-6-enylmagnesium bromide with tridec-12-en-2-one. It was converted by hydrogen iodide into (II) and thence by condensation with ethyl sodiomalonate into *ethyl* (1 : 11 : 17-*trimethylheneicosa*-11 : 17-*dienyl*)*malonate* [2 : 12 : 18-*trimethyldocosa*-12 : 18-*diene*-1 : 1-*dicarboxylate*] (III). Hydrolysis, followed by decarboxylation, afforded the dienoic acid (IV).

(I.) $CH_3 \cdot [CH_2]_3 \cdot CH{:}CMe \cdot [CH_2]_4 \cdot CH{:}CMe \cdot [CH_2]_3 \cdot CH{:}CH_2$
(II.) $CH_3 \cdot [CH_2]_3 \cdot CIMe \cdot [CH_2]_5 \cdot CIMe \cdot [CH_2]_3 \cdot CHIMe$
(III) $CH_3 \cdot [CH_2]_3 \cdot CH{:}CMe \cdot [CH_2]_4 \cdot CH \cdot CMe \cdot [CH_2]_3 \cdot CHMe \cdot CH(CO_2Et)_2$
(IV.) $CH_3 \cdot [CH_2]_3 \cdot CH{:}CMe \cdot [CH_2]_4 \cdot CH{:}CMe \cdot [CH_2]_3 \cdot CHMe \cdot CH_2 \cdot CO_2H$

Respecting the above condensation of (II) with ethyl sodiomalonate it should be noted that the tertiary carbon atoms bearing iodine are attached on each side to a methylene group so that the elimination of hydrogen iodide would be expected to occur in each of the two possible directions. Hence we are not entitled to assume that the resulting dienoic acid is homogeneous.

The preparation of 2-*methyltricosoic acid* and 2 : 12-*dimethyltricosoic acid* was then considered, in order to study the influence of omission of one or more of the methyl branches present in 2 : 12 : 18-trimethyltricosoic acid on the biological activity.

For the preparation of 2-methyltricosoic acid *docos*-1-*en*-12-*one* (V), obtained by the action of *n*-undecoyl chloride on undec-10-enylzinc iodide, was converted by hydrogen iodide into (VI); condensation with ethyl sodiomalonate, followed by hydrolysis and decarboxylation, gave 12-*keto*-2-*methyltricosoic acid* (VII) which by Clemmensen reduction afforded 2-methyltricosoic acid (VIII).

(V.) $CH_3 \cdot [CH_2]_9 \cdot CO \cdot [CH_2]_9 \cdot CH{:}CH_2$
(VI.) $CH_3 \cdot [CH_2]_9 \cdot CO \cdot [CH_2]_9 \cdot CHIMe$
(VII.) $CH_3 \cdot [CH_2]_9 \cdot CO \cdot [CH_2]_9 \cdot CHMe \cdot CH_2 \cdot CO_2H$
(VIII.) $CH_3 \cdot [CH_2]_{19} \cdot CHMe \cdot CH_2 \cdot CO_2H$

2 : 12-Dimethyltricosoic acid was synthesised by interaction of tridec-12-en-2-one with a Grignard solution from *n*-decyl bromide, to give 12-*methyldocos*-1-*en*-12-*ol* (IX) which by treat-

ment with hydrogen iodide, followed by condensation with ethyl sodiomalonate, afforded *ethyl* (1 : 11-*dimethylheneicos*-11-*enyl*)*malonate* (X). This was transformed in the usual stages into the required acid (XI).

(IX.) $CH_3 \cdot [CH_2]_9 \cdot C(OH)Me \cdot [CH_2]_9 \cdot CH{:}CH_2$

(X.) $CH_3 \cdot [CH_2]_8 \cdot CH{:}CMe \cdot [CH_2]_9 \cdot CHMe \cdot CH(CO_2Et)_2$

(XI.) $CH_3 \cdot [CH_2]_9 \cdot CHMe \cdot [CH_2]_9 \cdot CHMe \cdot CH_2 \cdot CO_2H$

Biological studies of the foregoing acids as well as those described in Part I were undertaken by Mr. C. E. Coulthard and Dr. L. Dickinson, of the Research Department of Boots Pure Drug Company, Ltd., and Dr. J. Ungar, of Glaxo Laboratories Ltd. Dr. Ungar states :

" The biological activity of these fatty acids has been studied in mice and guinea-pigs.

" In mice the acute toxicity has been controlled to determine the dose which can be safely injected without causing acute death. The substances have been injected in the form of homogeneous suspensions in 10% ethyl alcohol. In guinea-pigs the substances have been tested for their granuloma-producing properties. Different amounts of suspensions from 5 mg. to 200 mg. have been injected intraperitoneally into young guinea-pigs, and at regular intervals individual guinea-pigs were killed, and detailed *post-mortem* examinations performed. When the compounds were active we noticed after nine days the formation of small nodules, 2—3 mm. in diameter, on the omentum, and small similar nodules in the mesenterial glands and liver. After 14—18 days these nodules increased in number and size, and formed caseous lesions which greatly increased in size. After 4 weeks calcification of these nodules was already visible. In case of the more active compounds granuloma-like nodules were visible in the diaphragm and lungs. The histological sections showed pictures which resembled a specific granuloma, the primary cause of which seemed to be local necrosis. It started with a small cell infiltration with macrophages and development of epitheloid cells and giant cells of the Langerhans type." *

Of the substances described in this paper 2 : 12 : 18-trimethyltricosa-12 : 18-dienoic acid induces the above lesions when injected in a dose of 100 mg. 2 : 12-Dimethyltricosoic acid is slightly active in amounts of 50 mg. and 100 mg., and has some activity in a dose of 25 mg. 2-Methyltricosoic acid is inactive.

From these preliminary tests it is not yet possible to assess any differences in degree of activity and, in particular, further studies will be necessary to determine whether the ethylenic linkages in the above dienoic acid have any additional effect, as compared with the corresponding saturated acid.

The activity of 2 : 12-dimethyltricosoic acid seems to suggest that the $C_{(18)}$-methyl group of 2 : 12 : 18-trimethyltricosoic acid is not indispensable for the activity of the substance, but present evidence indicates that a third methyl branch, in addition to those at $C_{(2)}$ and $C_{(12)}$, is necessary in order to produce extensive lesions. Full discussion of this point must be reserved for a future communication.

The opportunity is taken to record in the Experimental section the preparations of 12 : 16-*dimethyltricosoic* and 12 : 15-*dimethyldocosoic acid*, the latter originally designed to serve as an intermediate for the preparation of its higher homologue, 13 : 16-dimethyltricosoic acid. These preparations were initiated in view of the high activity of a specimen of 12 : 15-dimethyltricosoic acid described in Part I. The work was discontinued when further investigations, to be described in a subsequent paper, indicated that this activity was probably due to the presence of a $C_{(2)}$-substituted isomer.

Experimental.

2 : 12 : 18-*Trimethyltricosa*-12 : 18-*dienoic Acid*.—12 : 18-*Dimethyldocosa*-1 : 12 : 18-*triene* (I). Ethyl 5-methyldec-5-enoate (55 g.; b. p. 125—130°), obtained by a Grignard reaction from *n*-butyl bromide and ethyl 5-ketoheptoate and dehydration of the resulting carbinol (cf. Polgar and Robinson, *loc. cit.*), was reduced by sodium (33 g.) and butyl alcohol (540 c.c.), to give 6-methyldec-6-enol (31 g.; 70% yield), b. p. 127—130°/17 mm., $n_D^{17°}$ 1·4556. This was dissolved in toluene (55 c.c.), and phosphorus tribromide (18 g., 6·3 c.c.) in toluene (55 c.c.) then added during 3 hours, with vigorous stirring while cooling in an ice–salt bath. Next day the mixture was decomposed by ice, and the bromide isolated in the usual way as a colourless oil (21 g.), b. p. 127°/17 mm., $n_D^{17°}$ 1·4780.

A solution of tridec-12-en-2-one (23·5 g.; Polgar and Robinson, *loc. cit.*) in ether (40 c.c.) was added to a Grignard compound from the above bromide (27 g.), magnesium (3·3 g.), and ether (50 c.c.), kept under nitrogen. The resulting carbinol, isolated in the usual manner, was dehydrated over a trace of iodine (170°; 2 hours) and gave a mobile oil (19 g.), a sample of which boiled, over sodium, at 176—185° (bath)/0·2 mm. affording 12 : 18-*dimethyldocosa*-1 : 12 : 18-*triene*, $n_D^{19°}$ 1·4720 (Found : C, 86·5; H, 13·5. $C_{22}H_{44}$ requires C, 86·65; H, 13·25%).

* *Added in Proof.*—Cf. *Brit. J. Exp. Path.*, 1948, **29**, 322.

Ethyl (1 : 11 : 17-*trimethylheneicosa*-11 : 17-*dienyl*)*malonate* (III). A solution of the crude diene (17·7 g.) in benzene (40 c.c.) was saturated with hydrogen iodide with cooling in ice. Next day the solvent was removed under diminished pressure at 30—40° (bath), and the residual oil added with stirring to an excess of ethyl sodiomalonate (from 7·5 g. of sodium and 70 g. of ethyl malonate) in alcohol (110 c.c.), while heating the mixture. It was kept overnight under nitrogen and then boiled under reflux for 3 hours, and the product isolated in the usual manner. After removal of substances, b. p. <150° (bath temperature)/9 mm., a viscous oil (26 g.) remained, a sample of which, on distillation, afforded *ethyl* (1 : 11 : 17-*trimethylheneicosa*-11 : 17-*dienyl*)*malonate* as a colourless oil, b. p. 206°/0·15 mm., $n_D^{17°}$ 1·4681 (Found : C, 75·7; H, 11·2. $C_{31}H_{56}O_4$ requires C, 75·6; H, 11·3%).

2 : 12 : 18-*Trimethyltricosa*-12 : 18-*dienoic acid* (IV). A sample (1·75 g.) of the foregoing malonic ester was hydrolysed by boiling it under reflux for 3 hours under nitrogen with a solution of potassium hydroxide (1 g.) in alcohol (25 c.c.), and the free acid decarboxylated on distillation in a high vacuum. This furnished 2 : 12 : 18-*trimethyltricosa*-12 : 18-*dienoic acid* as a viscous oil (1·4 g.), b. p. 210°/0·06 mm., $n_D^{19°}$ 1·4740 (Found : C, 79·1; H, 12·2. $C_{26}H_{48}O_2$ requires C, 79·5; H, 12·2%).

On hydrogenation of a sample (2·8 g.) of the above malonic ester in alcohol (100 c.c.) in the presence of Raney nickel, the theoretical amount of hydrogen ($2H_2$) was absorbed. The saturated product, hydrolysed and decarboxylated as usual, afforded 2 : 12 : 18-trimethyltricosoic acid (0·7 g.), b. p. 204°/0·25 mm., $n_D^{18°}$ 1·4590 (Found : C, 78·5; H, 12·9. Calc. for $C_{26}H_{52}O_2$: C, 78·8; H, 13·1%).

2-*Methyltricosoic Acid*.—*Docos*-1-*en*-12-*one* (V). Undec-10-enyl iodide (71 g.; b. p. 92°/0·24 mm., $n_D^{19°}$ 1·4960, obtained from undec-10-enol *via* the chloride by boiling the latter under reflux with sodium iodide in dry acetone) was added, according to the procedure of Blaise (*Bull. Soc. chim.*, 1911, [iv], **9**, pp. i—xxvi), to a mixture of toluene (41 c.c.), ethyl acetate (20 c.c.), and zinc–copper couple (37 g.), and the mixture heated under reflux for 5 hours. The mixture was then cooled with ice, and the solution decanted. A solution of undecoyl chloride (41 g.; obtained from *n*-undecoic acid by means of thionyl chloride) in toluene (25 c.c.) was introduced during 10 minutes with cooling and stirring, and the mixture decomposed by pouring it on ice. The *ketone*, isolated in the usual manner, distilled as a colourless liquid (23 g.), b. p. 190°/0·3 mm., which quickly solidified. After crystallisation from alcohol it had m. p. 53° (Found : C, 82·0; H, 13·1. $C_{33}H_{66}O$ requires C, 82·0; H, 13·1%).

Ethyl (11-*keto*-1-*methylheneicosyl*)*malonate*. The above ketone (21 g.) in benzene solution (60 c.c.) was treated in the usual way with hydrogen iodide, and the product condensed with ethyl sodiomalonate (from 3 g. of sodium, 27 c.c. of ethyl malonate, and 50 c.c. of alcohol), the mixture being heated under reflux for 5 hours. Next day the product was worked up and substances, b. p. <140°/20 mm., were removed by distillation. A portion (13·5 g.) of the residue was then distilled, affording *ethyl* (11-*keto*-1-*methylheneicosyl*)*malonate* (5 g.) as a viscous oil, b. p. 247°/0·1 mm. (Found : C, 71·9; H, 10·8. $C_{39}H_{74}O_5$ requires C, 72·2; H, 11·2%).

12-*Keto*-2-*methyltricosoic acid* (VII). This *acid* was obtained from the foregoing malonic ester by the usual successive stages (hydrolysis, decarboxylation) as a viscous oil (3·2 g.), b. p. 224°/0·1 mm., which quickly crystallised and had m. p. 64° after crystallisation from ethanol (Found : C, 75·4; H, 11·7. $C_{24}H_{46}O_3$ requires C, 75·4; H, 12·0%).

2-*Methyltricosoic acid* (VIII). To an alcoholic solution (57 c.c.) of the above keto-acid (3·1 g.) which had been saturated with hydrogen chloride was added amalgamated zinc (29 g.), and the mixture boiled under reflux for 20 hours. Further quantities of amalgamated zinc (20 g.) were then added, and the mixture was boiled under reflux for a further 24 hours, while repeatedly passing hydrogen chloride into the solution until saturation. After the excess of zinc had been removed by treatment with concentrated hydrochloric acid (50 c.c.), the mixture was poured into water and extracted with ether. On hydrolysis 2-*methyltricosoic acid* was obtained as a colourless crystalline solid, m. p. 63·5° after recrystallisation from methanol–ether (Found : C, 78·2; H, 13·1. $C_{24}H_{48}O_2$ requires C, 78·3; H, 13·1%). Its acetol ester, obtained by boiling the sodium salt with chloroacetone in alcoholic solution, furnished a *semicarbazone* (cf. Polgar, *Biochem. J.*, 1948, **42**, 207) which separated from alcohol as a white crystalline powder, m. p. 90° (Found : C, 70·2; H, 11·5; N, 8·5. $C_{28}H_{55}O_3N_3$ requires C, 69·85; H, 11·4; N, 8·7%).

2 : 12-*Dimethyltricosoic Acid*.—12-*Methyldocos*-1-*en*-12-*ol* (IX). Dodec-10-enoic acid, m. p. 18° (6 g.; obtained by reaction of a Grignard solution from undec-10-enyl iodide with solid carbon dioxide), was converted by thionyl chloride into its chloride, b.p. 150°/20 mm., which (6·4 g.) with methylzinc iodide afforded tridec-12-en-2-one (5 g.), b p. 150°/23 mm., $n_D^{23°}$ 1·4440 (another preparation of this ketone, from undec-10-enyl cyanide and methylmagnesium iodide, was described in Part I, *loc. cit.*). This ketone (7 g.) in ethereal solution (15 c.c.) was caused to react in the usual manner with a Grignard solution, prepared from *n*-decyl bromide (20 g.) and magnesium (2·7 g.) in ether (35 c.c.). The resulting *carbinol* distilled as a colourless viscous oil (8 g.), b. p. 160°/0·1 mm., $n_D^{20°}$ 1·4625 (Found : C, 81·9; H, 13·2. $C_{33}H_{66}O$ requires C, 81·7; H, 13·6%).

Ethyl (1 : 11-*dimethylheneicos*-11-*enyl*)*malonate* (X). The above carbinol (8 g.) was treated with hydrogen iodide in the manner already described, and the product condensed with ethyl sodiomalonate (from 21 c.c. of ethyl malonate and 2·2 g. of sodium in 40 c.c. of alcohol). *Ethyl* (1 : 11-*dimethylheneicos*-11-*enyl*)*malonate* was obtained as a colourless liquid (6·8 g.), b. p. 229°/0·1 mm., $n_D^{22°}$ 1·4612 (Found : C, 75·4; H, 11·6. $C_{30}H_{56}O_4$ requires C, 75·0; H, 11·7%).

2 : 12-*Dimethyltricosoic acid* (XI). The foregoing malonic ester (16·7 g.) was hydrogenated in alcoholic solution (34 c.c.) in the presence of palladised barium sulphate at room temperature and normal pressure. The saturated product afforded, by hydrolysis and decarboxylation of the free acid in the usual way, 2 : 12-*dimethyltricosoic acid* as a colourless oil (4·2 g.), b. p. 212—218°/0·7 mm. (Found : C, 79·0; H, 12·9. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The 2 : 4-*dinitrophenylsemicarbazone* of its acetol ester crystallised from a mixture of benzene and alcohol as a pale yellow powder, m. p. 125—127° (Found : N, 10·8. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

12 : 15-*Dimethyldocosoic Acid*.—11 : 14-*Dimethyleicos*-1-*en*-11-*ol*. Ethyl 1-methylheptylidenecyanoacetate [1-cyano-2-methylnon-1-enoate] [72 g.; obtained from methyl hexyl ketone and ethyl cyanoacetate in 88% yield by the method of Cope *et al.* (*J. Amer. Chem. Soc.*, 1941, **63**, 3452)], b. p. 159—

160°/12 mm., was hydrogenated (alcoholic solution, palladised strontium carbonate), and the reduced product hydrolysed as described previously (cf. Part I, section 5-Methyldecoic Acid, p. 393). Decarboxylation of the free acid afforded 2-methylnonoic acid (43 g.), b. p. 140°/12 mm., $n_D^{18°}$ 1·4362 (Found: C, 69·6; H, 11·6. Calc. for $C_{10}H_{20}O_2$: C, 69·8; H, 11·6%). The butyl ester (56 g.) of this acid on reduction with sodium and butyl alcohol gave 3-methylnonanol (28 g.), b. p. 110—117°/13 mm., which was converted into its bromide (29 g.), b. p. 110°/27 mm. The Grignard solution, prepared from this bromide (29 g.) and magnesium (3·5 g.) in ether (85 c.c.) was then brought to reaction with dodec-11-en-2-one (30 g.) in ether (50 c.c.) in the usual way, yielding 11 : 14-*dimethyleicos*-1-*en*-11-*ol* (18 g.) as a viscous oil, b. p. 175°/0·2 mm., $n_D^{22°}$ 1·4590 (Found: C, 81·0; H, 13·3. $C_{22}H_{44}O$ requires C, 81·5; H, 13·6%).

12 : 15-*Dimethyldocosoic acid.* The foregoing carbinol was dehydrated over iodine, as described in previous preparations, to the diene (16·2 g.), b. p. 164°/0·1 mm. Treatment with hydrogen bromide in the presence of benzoyl peroxide (0·1 g.) in toluene solution (160 c.c.), followed by condensation with ethyl sodiomalonate and the usual successive stages, afforded the unsaturated *acid* (8 g.), b. p. 202°/0·05 mm., $n_D^{18°}$ 1·4642 (Found: C, 78·4; H, 12·6. $C_{24}H_{46}O_2$ requires C, 78·6; H, 12·5%). This was esterified by heating it under reflux with methanol (100 c.c.) and acetyl chloride (5 c.c.; cf. Jakob and Freudenberg, *Ber.*, 1941, **74**, 1001), and the resulting methyl ester (7·9 g.), b. p. 197°/0·3 mm., hydrogenated in alcoholic solution (34 c.c.) in the presence of palladised barium sulphate. Hydrolysis gave the saturated acid, which was purified by converting it into its acetol ester and thence into the *semicarbazone*. This derivative was obtained as a white crystalline powder (3·2 g.), m. p. 70° after recrystallisation from alcohol (Found: C, 69·6; H, 11·4. $C_{28}H_{55}O_3N_3$ requires C, 69·7; H, 11·6%). The semicarbazone was then boiled under reflux with alcoholic potassium hydroxide, and the acidified product isolated by means of ether. Removal of the solvent gave 12 : 15-*dimethyldocosoic acid* which crystallised from methanol in long needles, m. p. 43° (Found: C, 78·8; H, 13·2. $C_{24}H_{48}O_2$ requires C, 78·3; H, 13·0%).

The portion of the above semicarbazone which remained in the mother-liquor from the crystallisation of the crude product was isolated by adding water and collecting the separated oil with ether. The free acid, obtained on hydrolysis, distilled as a viscous liquid (1·6 g.), b. p. 208°/0·6 mm. (Found: C, 77·4; H, 12·6%), which probably is mainly 2 : 11 : 14-trimethylheneicosoic acid, expected to arise by addition of hydrogen bromide to the diene in the previous stage, if it proceeds in accordance with Markownikow's rule.

12 : 16-*Dimethyltricosoic Acid.*—4-*Methyldecanol.* 3-Methyldecoic acid (Part I, *loc. cit.*) was converted into its *methyl* ester by boiling the acid (17·7 g.) under reflux with dry methanol (100 c.c.) and acetyl chloride (5 c.c.) for 6 hours. The isolated product (16 g., or 84%) had b. p. 111°/13 mm., $n_D^{13°}$ 1·4326 (Found: C, 72·0; H, 12·05. $C_{12}H_{24}O_2$ requires C, 72·0; H, 12·0%). Reduction of the ester by means of sodium and butanol afforded 4-*methyldecanol* (11 g.), b. p. 115°/9 mm., $n_D^{17°}$ 1·4420 (Found: C, 76·2; H, 14·0. $C_{11}H_{24}O$ requires C, 76·7; H, 14·0%).

Dimethylheneicos-1-*en*-11-*ol.* The above alcohol was converted into its bromide, b. p. 122°/14 mm., and a Grignard solution from the latter (11 g.) brought to reaction in the usual manner with dodec-11-en-2-one (10 g.). 11 : 15-*Dimethylheneicos*-1-*en*-11-*ol* was obtained as a viscous oil (4 g.), b. p. 170—173°/0·2 mm. (Found: C, 81·2; H, 13·5. $C_{23}H_{46}O$ requires C, 81·6; H, 13·6%).

12 : 16-*Dimethyltricosoic acid.* The foregoing carbinol was dehydrated over iodine (190°; 2 hours), and the crude diene then subjected to the treatment with hydrogen bromide in the presence of benzoyl peroxide, followed by condensation with ethyl sodiomalonate and the usual successive stages. The resulting unsaturated acid (2 g.), b. p. 200°/0·06 mm., furnished on hydrogenation (alcohol solution, palladised barium sulphate) 12 : 16-*dimethyltricosoic acid*, b. p. 202°/0·2 mm., $n_D^{18°}$ 1·4620 (Found: C, 78·0; H, 12·9. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The acetol ester of this acid gave on treatment with semicarbazide acetate an oil; it furnished a 2 : 4-*dinitrophenylsemicarbazone* as a pale yellow crystalline product, m. p. 130° after crystallisation from alcohol (Found: C, 63·5; H, 8·9. $C_{31}H_{59}O_7N_5$ requires C, 63·2; H, 8·5%).

Side-chain methyl determination of the acid by the Kuhn–Roth method gave 1·8 molecules of acetic acid; however, by analogy with the preceding preparation the acid probably contains a $C_{(2)}$-substituted isomer. The amount obtained was insufficient for further examination.

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329. *Methyl-substituted Long-chain Acids. Part III.*

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Previous studies indicated that 12 : 15-dimethyltricosoic acid (Part I, *J.*, 1945, 389) * exhibited some activity in stimulating the formation of pseudo-tuberculous lesions. It is now shown that this activity was due to the presence of a 2-methyl-substituted isomer of the acid. The substance, namely 2 : 11 : 14-*trimethyldocosoic acid*, is notably active, whereas a purified specimen of 12 : 15-dimethyltricosoic acid is found to be inactive.

In further experiments, designed to establish the essential structural features for the biological activity, 3 : 12 : 15-*trimethyltricosoic*, 2 : 12 : 15-*trimethyldocosoic*, and 1 : 11 : 14-*trimethyldocosoic acid* have been synthesised. All these acids are active.

2 : 11 : 14-*Trimethyldocos*-11-*enoic acid* appears to exhibit only weak activities.

PART I of this series (Polgar and Robinson, *loc. cit.*) recorded *inter alia* the preparation of 12 : 15-dimethyltricosoic acid.* The method of preparation involved at one stage the addition

* For numbering etc., see p. 1541.

of hydrogen bromide to an intermediate diene under peroxide-catalysed conditions, a process which may give rise to the formation, as a by-product, of a secondary bromide resulting from the addition of hydrogen bromide according to Markownikoff's rule. The acid exhibited some activity in producing lesions similar to those of tuberculosis, and the possibility that it may have contained some of its isomer, namely 2 : 11 : 14-trimethyldocosoic acid, suggested the examination of the last-mentioned acid, and also of a purified specimen of 12 : 15-dimethyltricosoic acid.

2 : 11 : 14-*Trimethyldocosoic acid* (I) was obtained by the procedure employed for the preparation of 12 : 15-dimethyltricosoic acid (Part I) with the modification that hydrogen iodide was used in place of hydrogen bromide, in which process there is no peroxide effect.

The purification of a specimen of 12 : 15-dimethyltricosoic acid, prepared by the procedure described in Part I, was accomplished by converting it into its acetol ester and thence into the semicarbazone (cf. Polgar, *Biochem. J.*, 1948, **42**, 207). After crystallisation of the latter from methanol, the pure acid was recovered by hydrolysis with hydrochloric acid in glacial acetic acid. The acid thus obtained melted at 40—42° (after crystallisation from methanol), whilst the original specimen had m. p. 14—15°.

Results of the biological tests with guinea-pigs, carried out by Mr. C. E. Coulthard, Dr. L. Dickinson, and Dr. J. Ungar, showed that the purified specimen of 12 : 15-dimethyltricosoic acid was inactive, whereas 2 : 11 : 14-trimethyldocosoic acid possessed considerable biological activity in respect of stimulating the formation of tuberculous-like tissue. Hence the activity of the original specimen of 12 : 15-dimethyltricosoic acid must have been due to the presence of 2 : 11 : 14-trimethyldocosoic acid.

(I.) $CH_3 \cdot [CH_2]_6 \cdot CHMe \cdot [CH_2]_3 \cdot CHMe \cdot [CH_2]_8 \cdot CHMe \cdot CH_2 \cdot CO_2H$

(II.) $CH_3 \cdot [CH_2]_6 \cdot CHMe \cdot [CH_2]_3 \cdot CHMe \cdot [CH_2]_8 \cdot CHMe \cdot [CH_2]_3 CO_2H$

(III.) $CH_3 \cdot [CH_2]_6 \cdot CHMe \cdot [CH_2]_2 \cdot CHMe \cdot [CH_2]_9 \cdot CHMe \cdot CH_2 CO_2H$

(IV.) $CH_3 \cdot [CH_2]_6 \cdot CHMe \cdot [CH_2]_2 \cdot CHMe \cdot [CH_2]_9 \cdot CHMe \cdot CO_2H$

In order to obtain further information as to the structural requirements for the biological activity, several other acids, closely related to 2 : 11 : 14-trimethyldocosoic acid, were prepared.

3 : 12 : 15-*Trimethyltricosoic acid* (II) was obtained from 2 : 11 : 14-trimethyldocosoic acid by Arndt–Eistert homologation.

2 : 12 : 15-*Trimethyldocosoic acid* was synthesised by interaction of tridec-12-en-2-one with a Grignard reagent from 3-methylnonyl bromide; treatment of the product with hydrogen iodide, followed by condensation with ethyl sodiomalonate and the usual successive steps, afforded the required acid (III).

For the synthesis of 1 : 11 : 14-*trimethyldocosoic acid* (IV) the general procedure followed for the previous acids was modified by using a bromo-ketone in place of the usual unsaturated one. The yield of 10 : 13-*dimethyleicos*-10-*enyl bromide*, obtained by the reaction between 3-methyldecylmagnesium bromide and 11-*bromundecan*-2-*one* was somewhat lower than in similar reactions with unsaturated ketones.

2 : 11 : 14-*Trimethyldocos*-11-*enoic acid* was prepared by the procedure described for the corresponding saturated acid, omitting the reduction of an unsaturated intermediate. It distilled with slight decomposition and failed to give satisfactory analytical figures.

In view of statements in the literature (cf. Buu-Hoï and Ratsimamanga, *Compt. rend. Soc. Biol.*, 1943, **137**, 189) that 1 : 1-dimethyl-substituted acids such as αα-dimethylstearic acid produce characteristic tuberculous tissue when injected into experimental animals, a specimen of this acid was prepared for comparison. The acid has been previously synthesised by Birch and Robinson (*J.*, 1942, 488), and by Buu-Hoï and Cagniant (*Z. physiol. Chem.*, 1943, **279**, 76); in both cases Haller's method was employed, which depends on the preparation of ω-trialkylacetophenones and their subsequent decomposition by sodamide. In the present work αα-dimethylstearic acid was prepared from methyl α-methylstearate by interaction with sodium triphenylmethyl and methyl iodide (cf. Hudson and Hauser, *J. Amer. Chem. Soc.*, 1941, **63**, 3156; Polgar and Robinson, *J.*, 1943, 615). The acid was found to be biologically inactive.

Dr. J. Ungar summarises the physiological action of the acids studied as follows:

12 : 15-Dimethyltricosoic acid (original specimen, probably containing 2 : 11 : 14-trimethyldocosoic acid	weak at 100 mg.
2 : 11 : 14-Trimethyldocosoic acid	active at 25 mg.
2 : 12 : 15-Trimethyldocosoic acid	active at 25 mg.
1 : 11 : 14-Trimethyldocosoic acid	active at 25 mg.
3 : 12 : 15-Trimethyltricosoic acid	active at 50 mg.
2 : 11 : 14-Trimethyldocos-11-enoic acid	weak at 100 mg.
αα-Dimethylstearic acid	inactive

Dr. Ungar states in respect of the foregoing table:

"The classification is purely arbitrary and as a criterion of potency we used the minimum quantity of fatty acid, which, when injected intraperitoneally in guinea-pigs, caused granulomatous lesions macroscopically similar to lesions caused by tubercle bacilli or similar organisms."

It is not possible at this stage to define precisely the structural features essential for the biological activity, since many points remain to be determined. For example, whether acids with all three methyl branches near the middle of the chain, such as 9 : 12 : 15-trimethyltricosoic acid, are active, is as yet unknown.

From comparison of the acids studied it appears that, with two of the methyl branches at about $C_{(11)}$ and $C_{(14)}$, it makes little or no difference whether the third branch is at $C_{(1)}$, $C_{(2)}$, or $C_{(3)}$; however, it may be mentioned that the activity of 3 : 12 : 15-trimethyltricosoic acid is to be treated with some reserve, since its mode of preparation does not preclude the possibility of its being contaminated with some of the active lower homologue (2 : 11 : 14-trimethyldocosoic acid).

Comparison of the acids described in Part II indicates that acids with only two methyl branches, of which one is near the carboxyl group, such as 2 : 12-dimethyltricosoic acid, are slightly active. On the other hand, a purified specimen of 12 : 15-dimethyltricosoic acid (both methyl branches in the middle of the chain) was found inactive. Hence it may be concluded that three methyl branches are necessary in order to produce extensive lesions, and one of these must be near the carboxyl group. As to the other two branches, it may be stated that both 2 : 12 : 15-trimethyldocosoic acid and 2 : 12 : 18-trimethyltricosoic acid are active, although differences in the degree of activity of these acids may exist; there are no examples yet available of acids with all the three methyl branches near the carboxyl group.

Another point of interest emerging from these studies is that 1 : 11 : 14-trimethyldocosoic acid is active, whereas 1 : 12 : 16 : 20-tetramethyldocosoic acid (cf. Part I) was found to be inactive. The constitutional features distinguishing the last-mentioned acid are the presence of the $C_{(20)}$-methyl, further methyl branches at $C_{(12)}$ and $C_{(16)}$ (as compared with $C_{(11)}$ and $C_{(14)}$, or $C_{(12)}$ and $C_{(15)}$, and $C_{(12)}$ and $C_{(18)}$, respectively, in case of the active acids), and also the fact that it has been synthesised from an optically active starting material [(+)-citronellal]. In order to determine the cause of the biological inactivity further investigation is necessary.

The comparison of 2 : 11 : 14-trimethyldocos-11-enoic acid with the corresponding saturated acid seems to suggest that the introduction of an olefinic linkage results in a distinct diminution of the biological potency. It is to be noted, however, that there seems to be no diminution of activity in the case of 2 : 12 : 18-trimethyltricosa-12 : 18-dienoic acid (cf. Part II), as compared with 2 : 12 : 18-trimethyltricosoic acid.

The methyl substituent near to the carboxyl group may plausibly be assumed to function as protection against destruction by β-oxidation. Thus the acid containing it may have a better survival value and a more persistent effect.

The most likely function of the more remote methyl groups is to promote dehydrogenation.

Both these hypotheses are speculative, but are mentioned because they do afford some guidance in the selection of acids which are to be synthesised and biologically tested.

Experimental.

αα-Dimethylstearic Acid.—Methyl α-methylstearate (b. p. 143—147°/0·1 mm.; acetol ester semicarbazone of the acid, m. p. 86—88°) (10 g.) was methylated by means of sodium triphenylmethyl (from 14 g. of triphenylchloromethane and 150 g. of 1·5% of sodium amalgam in 120 c.c. of ether) and methyl iodide (6 g.). The resulting ester was hydrolysed, and the isolated crude acid neutralised with alcoholic sodium hydroxide (phenolphthalein) and esterified by heating it under reflux with an excess of chloroacetone. The resulting acetol ester gave a *semicarbazone*, m. p. 66—68° (from methanol) (Found: C, 67·6; H, 11·2. $C_{24}H_{47}O_3N_3$ requires C, 67·75; H, 11·05%) (yield, 7 g.). The semicarbazone (6 g.) was heated on the steam-bath with acetic acid and hydrochloric acid as described below for the purification of 12 : 15-dimethyltricosoic acid, but the product obtained on distillation did not solidify when cooled with ice-water; it was subsequently boiled for one hour with 10% aqueous methanolic potassium hydroxide. Isolation and distillation of the acid gave an almost colourless oil, b. p. 177—180°/0·1 mm., which easily solidified and melted at 46—48°; the m. p. was raised to 48—49° after one crystallisation from methanol (Buu-Hoï and Cagniant, *loc. cit.*, crystallised this acid from light petroleum and give m. p. 50—51°, but very considerable losses of material occurred when this solvent was used).

12 : 15-*Dimethyltricosoic Acid.*—This acid (9 g.; prepared by the procedure described by Polgar and Robinson, *loc. cit.*) was converted into the semicarbazone of its acetol ester in the manner already described; after crystallisation from methanol, the semicarbazone was obtained as colourless crystals, m. p. 70—71° (4·2 g.) (Found: C, 70·3; H, 11·4; N, 8·8. Calc. for $C_{33}H_{67}O_3N_3$: C, 70·3; H, 11·5; N, 8·5%). The semicarbazone (4 g.) was heated with glacial acetic acid (17 c.c.) and concentrated hydrochloric acid (3 c.c.) on the steam-bath; the solution darkened and an oil separated on the surface. After one hour

the mixture was cooled, diluted with water (40 c.c.), and extracted with light petroleum (b. p. 40—60°). Distillation gave a colourless oil, b.p. 203—205°/0·3 mm., which solidified on cooling and separated from hot methanol as colourless scales (2·6 g.), m. p. 40—42°.

2 : 11 : 14-*Trimethyldocosoic Acid* (I).—11 : 14-Dimethylheneicosa-1 : 11-diene (10 g.) was added to an ice-cold, saturated solution of anhydrous hydrogen iodide in dry benzene (80 c.c.); after being kept over-night, the solution was evaporated under reduced pressure in a bath at 35—40°. The residue was then added to a solution of ethyl malonate (25 g.) and sodium (2·5 g.) in alcohol (40 c.c.), and the mixture heated under reflux for 4 hours. *Ethyl* 1-*carbethoxy*-2 : 11 : 14-*trimethyldocos*-11-*enoate* (10 g.) was thus obtained as a pale yellow oil, b. p. 212—216°/0·15 mm., $n_D^{19°}$ 1·4612 (Found: C, 74·6; H, 11·6. $C_{30}H_{56}O_4$ requires C, 75·0; H, 11·7%). Hydrogenation of this ester (9·5 g.) with palladised strontium carbonate in alcohol at the atmospheric pressure, followed by alkaline hydrolysis and decarboxylation, gave 2 : 11 : 14-*trimethyldocosoic acid*, a colourless oil, b. p. 201—203°/0·2 mm.; on redistillation it had b. p. 189—191°/0·02 mm., $n_D^{18°}$ 1·4616 (Found: C, 78·4; H, 13·0. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The 2 : 4-*dinitrophenylsemicarbazone* of its acetol ester was a crystalline powder (from alcohol) which became sticky when filtered, and melted at 128—132° after sintering at 110° (Found: N, 10·4. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

2 : 11 : 14-*Trimethyldocos*-11-*enoic Acid*.—Ethyl 1-carbethoxy-2 : 11 : 14-trimethyldocos-11-enoate (see preceding section) was hydrolysed, and the free acid decarboxylated in the usual manner. The product, which was an oil, could not be distilled without decomposition. It was esterified with ethanol and acetyl chloride, and the ester distilled; b. p. 188—192°/0·15 mm. On hydrolysis the unsaturated acid was obtained as a colourless liquid, b. p. 201—204°/0·1 mm., $n_D^{16°}$ 1·4662, which failed to give satisfactory figures on analysis (Found: C, 78·0, 77·8; H, 12·3, 12·4. $C_{25}H_{48}O_2$ requires C, 78·95; H, 12·6%).

3 : 12 : 15-*Trimethyltricosoic Acid* (II).—2 : 11 : 14-Trimethyldocosoic acid (3 g.) was converted into its chloride by means of phosphorus trichloride (0·5 c.c.) and a drop of pyridine in benzene solution; the solution was decanted and evaporated under reduced pressure, and a solution of the residue in ether mixed with an ethereal solution of diazomethane (from 10 g. of nitrosomethylurea). Next day the excess of the reagent and the ether were evaporated (bath at 50°), finally under diminished pressure. The residue was dissolved in dry methanol (30 c.c.) and decomposed on the steam-bath by the addition of silver oxide (from 4 c.c. of a 10% solution of silver nitrate) during one hour. The filtered solution was heated under reflux with 10% potassium hydroxide (20 c.c.) for 30 minutes. The *acid*, isolated by means of ether, was a colourless oil, b. p. 198—200°/0·3 mm., $n_D^{18°}$ 1·4599 (Found: C, 78·9; H, 13·4. $C_{26}H_{52}O_2$ requires C, 78·8; H, 13·1%) (yield, 1·6 g.). The 2 : 4-*dinitrophenylsemicarbazone* of the acetol ester of this acid sintered at 110° and melted at 129—132° (Found: N, 9·9. $C_{36}H_{61}O_7N_5$ requires N, 10·3%).

1 : 11 : 14-*Trimethyldocosoic Acid* (IV).—ω-Hydroxydecoic acid was obtained by the procedure of Grün and Wirth (*Ber.*, 1922, **55**, 2206) with the following modifications in detail. The temperature during the addition of potassium permanganate was kept at about 50°; when the oxidation had been completed, the mixture was diluted with twice its volume of water, and the manganese sludge dissolved by passing in sulphur dioxide. In this way the yield was increased to 90%, based upon the undec-10-enol employed as starting material. The hydroxy-acid was then converted into ω-bromodecoic acid by heating it under reflux with concentrated hydrobromic and sulphuric acid (Chuit and Hausser, *Helv. Chim. Acta*, 1929, **12**, 474, used a 50% solution of hydrogen bromide in glacial acetic acid).

The action of ω-bromodecoyl chloride on methylzinc iodide (Blaise method) afforded 11-*bromundecan*-2-*one* in excellent yield. It was a colourless liquid, b. p. 148—152°/8 mm., $n_D^{18°}$ 1·4692 (Found: C, 53·5; H, 8·0. $C_{11}H_{21}OBr$ requires C, 53·0; H, 8·4%). Its 2 : 4-*dinitrophenylhydrazone* sintered at 82° and melted at 89—91° (Found: N, 13·5. $C_{17}H_{25}O_4N_4Br$ requires N, 13·05%).

The foregoing ketone (10 g.) was mixed with a Grignard solution from 3-methyldecyl bromide (12 g.) and magnesium (1·2 g.). Dehydration of the product by heating it with a crystal of iodine, followed by distillation, gave, after separation of some lower boiling fractions, 10 : 13-*dimethyleicos*-10-*enyl bromide* as a colourless liquid, b. p. 170—176°/0·2 mm.; on redistillation it had b. p. 172—174°/0·2 mm., $n_D^{19°}$ 1·4758 (Found: C, 67·8; H, 11·2. $C_{22}H_{43}Br$ requires C, 68·2; H, 11·1%) (yield, 8·5 g.).

This unsaturated bromide (8 g.) was boiled under reflux for 6 hours with a solution of ethyl methylmalonate (10·5 g.) and sodium (1·4 g.) in absolute alcohol (30 c.c.). After the product had been worked up in the usual way, *ethyl* 1-*carbethoxy*-1 : 11 : 14-*trimethyldocos*-11-*enoate* (8 g.) was obtained as an almost colourless oil, b. p. 206—210°/0·05 mm., and this, on hydrogenation (palladised strontium carbonate as catalyst), hydrolysis, and decarboxylation, afforded 1 : 11 : 14-*trimethyldocosoic acid* (5·7 g.), b. p. 201—203°/0·08 mm., $n_D^{17°}$ 1·4572 (Found: C, 78·7; H, 13·25. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The 2 : 4-*dinitrophenylsemicarbazone* of its acetol ester, crystallised from alcohol containing a little light petroleum (b. p. 60—80°), had m. p. 136—138° (sintered at 120°) (Found: N, 11·0. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

2 : 12 : 15-*Trimethyldocosoic Acid* (III).—Tridec-12-en-2-one was prepared by the Blaise method, which afforded a somewhat shorter route than that used in a previous preparation (Polgar and Robinson, *loc. cit.*). Zinc–copper couple (50 g.), toluene (25 c.c.), ethyl acetate (12 c.c.), and methyl iodide (5 c.c.), with a trace of iodine, were heated on the steam-bath until a reaction began; undecenyl iodide (50 g.) was then added in 4 portions during one hour. Heating on the steam-bath was continued for 3 hours, and the mixture then gently boiled under reflux in an oil-bath at 120° for a further 3 hours. After cooling with ice-water, the solution was decanted, the residual zinc–copper couple rinsed with toluene (25 c.c.), and this toluene added to the decanted solution. Freshly distilled acetyl chloride (14 c.c.) was then slowly added in small portions with cooling (ice-water) and shaking, and the product worked up in the usual way. Yield, 26·8 g.

The above ketone (9·8 g.) was added to a Grignard solution from 3-methylnonyl bromide (13 g.). Distillation of the product gave 11 g. of a colourless liquid, b. p. 152—155°/0·25 mm., whose analysis showed it to consist of a mixture of a diene and the corresponding unsaturated carbinol. This mixture was submitted to the addition of hydrogen iodide and the malonic ester synthesis in the manner already described, and gave a liquid which could not be distilled without decomposition. It was, therefore,

hydrolysed and decarboxylated, and the product re-esterified by boiling it with a mixture of ethanol (20 c.c.) and acetyl chloride (2 c.c.). *Ethyl* 2 : 12 : 15-*trimethyldocos*-12-*enoate* (6 g.), b. p. 185—187°/0·25 mm., $n_D^{20°}$ 1·4585 (Found : C, 79·6; H, 12·65. $C_{27}H_{52}O_2$ requires C, 79·4; H, 12·75%), was thus obtained; this, when reduced catalytically (palladised strontium carbonate) and then hydrolysed, yielded 4·2 g. of 2 : 12 : 15-*trimethyldocosoic acid*, b. p. 202—204°/0·3 mm., $n_D^{18°}$ 1·4562 (Found : C, 78·8; H, 12·9. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). Its acetol ester gave a 2 : 4-*dinitrophenylsemicarbazone*, m. p. 128—130° (sintered at 115 (Found : N, 10·6. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

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330. *A Synthesis of Acylamidomalondialdehydes.*

By J. W. CORNFORTH, (MISS) E. FAWAZ, L. J. GOLDSWORTHY, and SIR ROBERT ROBINSON.

Oxazole-4-aldehydes are converted by aqueous alkali into acylamidomalondialdehydes, which are intermediates in certain schemes for the synthesis of penicillins.

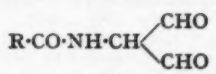
IT is noteworthy that penicillin syntheses, by which term we mean not only the proved synthesis of benzylpenicillinic acid, but also the production of traces of biologically active material destroyed by penicillinase, have been demonstrated only by way of an oxazolone. For example, the various claims that penicilloic acids have been dehydrated to penicillins are of doubtful validity.

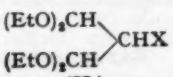
There is even no evidence that inactivated penicillin can be activated *in vivo*. In considering possible alternative last stages of the biogenesis of penicillin, an oxidative process is attractive. Thus a four-membered ring containing :N–CH(OH)– might be initially produced from :NH OCH–, more easily than is :N–CO– from :NH CO_2H–, and then suffer oxidation.

An attempt to realise this scheme led to the present work.

Attempts to prepare acylamidomalondialdehydes (I) by application of conventional methods were uniformly unsuccessful. For example, hippuraldehyde could not be formylated, and the halogen atom in *chloromalondialdehyde tetraethyl acetal* (II; X = Cl) proved to be too little reactive for smooth replacement by amines, though *benzylaminomalondialdehyde tetraethyl acetal* (II; X = $CH_2Ph·NH$) could be prepared in low yield, and some indication of formation of amino-acetal (II; X = NH_2) was found after heating it with alcoholic ammonia to a high temperature.

Earlier work at Oxford (see "The Chemistry of Penicillin," Chap. XXI) had made 2-substituted oxazole-4-aldehydes (III) readily available. An examination of these aldehydes led to the rather surprising discovery that on warming them with dilute alkali the oxazole ring underwent fission with formation of acylamidomalondialdehydes in excellent yield.


(I.)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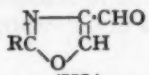
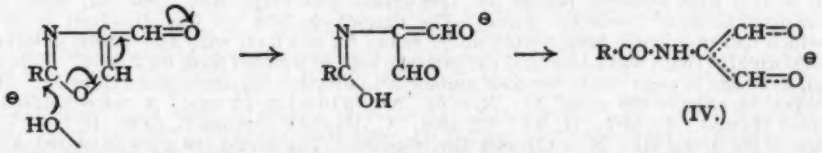

(III.)

The oxazole ring usually has great stability towards alkali; 4-phenyl-2-methyloxazole, for example, resists the action of alcoholic potassium hydroxide at 200° and can be distilled unchanged over hot soda-lime (Lewy, *Ber.*, 1888, **21**, 924). The ready fission of these aldehydes probably involves attack on C_2 by a hydroxyl ion, the driving force of the reaction being derived from the "resonance" stabilisation of the symmetrical malondialdehyde anion (IV). The process is analogous to a rearrangement of certain oxazoles described elsewhere ("The Chemistry of Penicillin," Chap. XXI) and thought to involve fission and recyclisation at C_2.

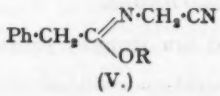

(IV.)

Oxazole-4-aldehydes are available from the corresponding carboxylic acid chlorides and nitriles by the Rosenmund and the Stephen reduction, respectively. A synthesis of 4-cyano-oxazoles has already been described (Cornforth and Huang, *J.*, 1948, 1969). Oxazole-4-carboxlic

acids can be made by analogous procedures (Cornforth and Cornforth, *J.*, 1947, 96) or alternatively by rearrangement of 4-hydroxymethyleneoxazolones (*op. cit.*, Chap. XXI).

Phenylacetimido methyl ether and the related ethyl ether reacted with aminoacetonitrile to give 1-*methoxy-* and 1-*ethoxy-2-phenylethylideneaminomethyl cyanide* (V). These product were formylated with ethyl formate–potassium ethoxide, and the resulting potassium salts (VI) added to boiling acetic acid, giving *4-cyano-2-benzyloxazole* (VII). The condensation with aminomethyl cyanide was more satisfactory with phenylacetimido ethyl ether, but formylation of (V; R = Me) was easier, the potassium salt crystallising much more readily.

The potassium salt (VI; R = Me) reacted with ammonium sulphate to form *4-cyano-2-benzylglyoxaline* (VIII) (cf. Cornforth and Cornforth, *loc. cit.*). This substance was amphoteric, ionisation of the imino-hydrogen atom being evidently favoured by the electron-attracting cyano-group.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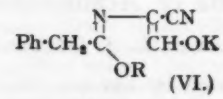
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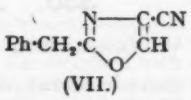
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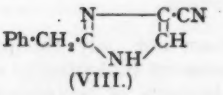
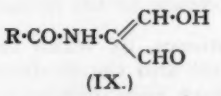

(VIII.)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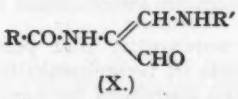
(X.)

Reduction of the cyanide (VII) to *2-benzyloxazole-4-aldehyde* (III; R = CH_2Ph) by Stephen's method proceeded smoothly, though it was necessary to decompose the aldimine stannichloride with sodium hydrogen carbonate.

Reduction of 2-phenyloxazole-4-carboxyl chloride by Rosenmund's technique gave *2-phenyloxazole-4-aldehyde* (III; R = Ph), and the same method was used to prepare 2-benzyloxazole-4-aldehyde from *2-benzyloxazole-4-carboxyl chloride* (or its 5-chloro-derivative).

Alkaline fission of the appropriate oxazole-aldehyde gave *benzamido-*, *phenylacetamido-*, and *hexamido-malondialdehydes* (I or IX; R = Ph, CH_2Ph, or *n*-C_5H_{11}). These substances were crystalline solids and could be kept for long periods without deterioration. They resembled known malondialdehydes in most properties and reactions. The ultraviolet light absorption of phenylacetamidomalondialdehyde indicated that the enol form (IX; R = CH_2Ph) was predominant. Condensation of the dialdehydes with primary amines gave derivatives (X) of αβ-diaminoacraldehyde.

Condensation of the acylamidomalondialdehydes, especially the phenylacetamido-derivative, with D-penicillamine and its methyl ester was studied in a preliminary way at Oxford and the bisthiazolidine derivative was isolated without difficulty. In experiments giving variable results, indications of antibiotic activity, partly destroyed by penicillinase, were obtained.

These findings are not described in the present paper because the investigation has been transferred to Hampstead, and it is hoped that one of us (J. W. C.) will be able to carry the matter a stage further.

Experimental.

Chloromalondialdehyde.—The process follows the indications given by Prins [D.R.-P. 261689 (1913)]. 1 : 1 : 2 : 3 : 3-Pentachloropropane (54 g.; *Rec. Trav. chim.*, 1935, **54**, 307) was stirred and treated below 20° with a solution of potassium hydroxide (15 g.) in ethanol (100 c.c.), added during one hour. After a further ½ hour, water (500 c.c.) was added, and 1 : 2 : 3 : 3-tetrachloropropene (45 g.) was isolated by means of ether; b. p. 54°/15 mm. Tetrachloropropene (25 g.) was mixed with sulphuric acid (75 c.c.; *d* 1·84) and stirred rapidly at 40—50° until evolution of hydrogen chloride ceased (8 hours). The mixture was poured into ice and water (100 c.c.), and the precipitate (8 g.) collected. Continuous extraction of the filtrate with ether (10 hours) gave a further 2 g. Recrystallisation of the whole product from benzene containing a few drops of acetone gave chloromalondialdehyde in colourless prisms, m. p. 145—146°. A further quantity could be obtained from the aqueous mother-liquor by precipitation as the anil and hydrolysis of this with alcoholic potash (cf. Dieckmann and Platz, *Ber.*, 1904, **37**, 4638).

Chloromalondialdehyde Tetraethyl Acetal.—The dialdehyde (5·4 g.) was dissolved in dry ethanol (15 c.c.) which had previously been heated under reflux for one hour with ammonium chloride (0·5 g.). Ethyl orthoformate (16 g.) was added and the mixture kept in a closed flask for 5 days. Ether (100 c.c.) was added, and then enough dilute aqueous ammonia to dissolve the ammonium chloride. Distillation of the ethereal layer gave the *acetal* (II; X = Cl), b. p. 116—120/12 mm. A twice-distilled specimen was analysed (Found: C, 51·7; H, 9·1; Cl, 13·8. $C_{11}H_{23}O_4Cl$ requires C, 52·0; H, 9·0; Cl, 13·8%).

Reaction of the Acetal (II; X = Cl) *with Benzylamine.*—The acetal (10 g.) was heated under reflux for 12 hours with benzylamine (20 c.c.). After dilution with ether (200 c.c.) and separation of benzylamine hydrochloride, ether and benzylamine were removed by distillation at low pressure. The residue, dissolved in ether (100 c.c.), was shaken at 0° with dilute sulphuric acid (2 × 15 c.c.), which was separated, quickly neutralised with sodium carbonate, and extracted repeatedly with ether. The

ether-soluble product (3 g.) was distilled, and the fraction, b. p. 80—85°/0·5 mm., containing *benzylaminomalondialdehyde tetraethyl acetal* (II; X = Ph·CH_2·NH), collected and redistilled (Found: C, 66·0; H, 9·5; N, 4·8. $C_{18}H_{31}O_4N$ requires C, 66·4; H, 9·5; N, 4·3%). The picrate could not be purified. The acetal with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid gave, after neutralisation, a product, probably *benzylaminomalondialdehyde mono-2 : 4-dinitrophenylhydrazone*, which had m. p. 211—213° (decomp.) after crystallisation from ethanol (Found: C, 54·1; H, 3·7. $C_{16}H_{15}O_8N_5$ requires C, 53·8; H, 4·1%).

Reaction of the Acetal (II; X = Cl) *with Ammonia.*—The acetal (6·3 g.), dry methanol (100 c.c.), and dry ammonia (28 g.) were heated in an autoclave at 150° for 10 hours. The mixture was concentrated to 50 c.c., 5% potassium hydroxide (10 c.c.) added, and evaporation continued until the vapour no longer burned. The supernatant oil, consisting largely of unchanged acetal, was separated. Extraction of the aqueous liquor, after saturation with sodium chloride, for ten hours with ether gave an oil (0·5 g.) which gave a precipitate with picric acid in ethanol. The oil (0·59 g.) obtained from a similar experiment conducted at 200° was distilled in bulbs. One fraction appeared to contain the desired *aminomalondialdehyde tetraethyl acetal* (II; X = NH_2) (Found: C, 55·7; H, 10·9; N, 5·0. $C_{11}H_{25}O_4N$ requires C, 56·2; H, 10·6; N, 5·9%).

1-*Methoxy- and* 1-*Ethoxy-2-phenylethylideneaminomethyl Cyanide* (V; R = Me and Et).—(i) Phenylacetimido methyl ether (90 g.) was added to a chilled mixture of saturated aqueous sodium hydrogen carbonate (500 c.c.) and ether (100 c.c.). After swirling and separating, the aqueous layer was extracted with ether (50 c.c.) both before and after being made alkaline to phenolphthalein. The ethereal extracts were dried at −10° (K_2CO_3) and distilled, giving phenylacetimido methyl ether (61·5 g.; 85%). On cooling, this crystallised in large prisms, m. p. 32°.

Aminomethyl cyanide hydrogen sulphate (20 g.) in water (10 c.c.) was neutralised to methyl-orange with concentrated potassium hydroxide solution. A further 5 g. of the sulphate was then added, followed by a solution of phenylacetimido methyl ether (25 g.) in ether (125 c.c.). The mixture was cooled in ice and stirred vigorously for half an hour; the ether was separated, washed with a little water, and dried quickly ($MgSO_4$). After removal of ether at room temperature, the residue was distilled. A fraction (10·8 g.) boiling below 80°/0·1 mm. was methyl phenylacetate containing some unchanged imido-ether. The main fraction (13·8 g.), a colourless oil, b. p. 100°/0·1 mm., was the desired 1-*methoxy-2-phenylethylideneaminomethyl cyanide* (V; R = Me). It was fairly stable in closed vessel at −10°, but quickly reddened at room temperature (Found: C, 69·5; H, 6·5. $C_{11}H_{12}ON_2$ requires C, 70·2; H, 6·3%).

In the preparation of aminomethyl cyanide hydrogen sulphate (Anslow and King, *J.*, 1929, 2465) a mixture of acid and neutral sulphates was often obtained. When this happened, the composition was determined by titration with alkali, after which the weight of salt and the degree of neutralisation necessary could be calculated.

(ii) Phenylacetimido ethyl ether (7 g.) was diluted with a little ether, cooled to −10° and added to a similarly-cooled, concentrated solution of aminomethyl cyanide hydrochloride (4 g.) in water. After being shaken for 2½ hours and kept at 0° over-night, the ethereal layer was separated, and the aqueous layer extracted with ether. Isolation of the product then followed as above; the colourless oily product (6·3 g.), 1-*ethoxy-2-phenylethylideneaminomethyl cyanide* (V; R = Et), had b. p. 125°/0·5 mm. (Found: C, 70·8; H, 7·1; N, 13·7. $C_{12}H_{14}ON_2$ requires C, 71·3; H, 6·9; N, 13·9%).

4-*Cyano-2-benzyloxazole.*—Potassium (1·05 g.) was dissolved in alcohol (4 c.c.) and ether (15 c.c.). The solution was diluted with ether to 100 c.c., cooled to −10°, and treated with a mixture of 1-methoxy-2-phenylethylideneaminomethyl cyanide (5 g.) and ethyl formate (3 c.c.). After being kept at <0° for two hours and then overnight in a refrigerator, the crystalline, hygroscopic potassium salt (VI; R = Me) was collected, washed quickly with ether, and dried in a vacuum (yield, 5·6 g.). It showed with alcoholic ferric chloride the colour changes characteristic of its class (Cornforth and Cornforth, *loc. cit.*).

The salt was added to boiling acetic acid (15 c.c.) during five minutes. Water and alkali were added and the product extracted with ether. On distillation, 4-*cyano-2-benzyloxazole* (VII) (3·3 g.), b. p. 120°/0·2 mm., was obtained; it had a tendency to remain supercooled, but eventually solidified. Crystallisation from ether gave colourless prisms, m. p. 59° (Found: C, 71·4; H, 4·2; N, 15·6. $C_{11}H_8ON_2$ requires C, 71·1; H, 4·3; N, 15·2%).

The formylation of 1-ethoxy-2-phenylethylideneaminomethyl cyanide was carried out in similar fashion, but the potassium salt separated more slowly and tended to be gelatinous.

4-*Cyano-2-benzylglyoxaline.*—A crude sample of the potassium salt (VI; R = Me) was dissolved in water and an excess of ammonium sulphate was added. After several hours the mixture was neutralised to litmus and extracted with ethyl acetate, which was then extracted with dilute hydrochloric acid. Neutralisation of the chilled acid extract, and crystallisation of the precipitate from water (charcoal), gave the *glyoxaline* (VIII) in long colourless needles, m. p. 192·5—194° (Found: C, 71·9; H, 4·8; N, 22·8. $C_{11}H_9N_3$ requires C, 72·1; H, 4·9; N, 22·9%). The substance was soluble in dilute acids (including acetic) and also in dilute sodium hydroxide, but not in cold aqueous sodium carbonate.

2-*Benzyloxazole-4-carboxyl Chloride.*—The synthesis of 2-benzyloxazole-4-carboxylic acid from phenylacetimido ethyl ether was reported, without details of procedure, by Merck and Co. "The Chemistry of Penicillin, Chap. XXI). Ethyl 1-ethoxy-2-phenylethylideneaminoacetate was prepared from the imido-ether and glycine ethyl ester hydrochloride, using the technique described above for the condensation of the same imido-ether with aminomethyl cyanide hydrochloride; the ester (20 g. from 16·5 g. of imido-ether) was a colourless oil, b. p. 130—135°/0·5 mm. (Found: C, 68·1; H, 7·6. Calc. for $C_{14}H_{19}O_3N$: C, 67·5; H, 7·6%). Formylation in the usual manner and cyclisation of the resulting potassium salt with hydrogen chloride in alcohol–ether gave ethyl 2-benzyloxazole-4-carboxylate, m. p. 74—75°. This was hydrolysed by boiling under reflux for 10 minutes with a 10% excess of 15% aqueous potassium hydroxide. The cooled solution was stirred and treated dropwise with the calculated amount of N-sulphuric acid. The precipitated 2-benzyloxazole-4-carboxylic acid was collected; m. p. 156—158°.

A solution of thionyl chloride (5·5 c.c.) in pure chloroform (33 c.c.) was added to a suspension of the acid (8·9 g.) in the same solvent (22 c.c.). After the mixture had been heated under reflux for two hours

and evaporated at low pressure, the product was crystallised from light petroleum (b. p. 60—80°). 2-*Benzyloxazole-4-carboxyl chloride* (9 g.) formed long prisms, m. p. 62° (Found: C, 59·6; H, 3·7; N, 6·3. $C_{11}H_8O_2NCl$ requires C, 59·6; H, 3·6; N, 6·3%).

2-*Benzyloxazole-4-aldehyde*.—(i) Hydrogen was bubbled through a boiling solution of 2-benzyloxazole-4-carboxyl chloride (2·4 g.) in xylene (8 c.c.), containing palladised barium sulphate (0·65 g.) in suspension. The evolution of hydrogen chloride was substantially complete after 2½ hours. Catalyst and solvent were removed, and the residue was crystallised from light petroleum (b. p. 60—80°). The *aldehyde* (1 g.), thin prisms, had m. p. 70—71°, raised to 72° by recrystallisation (Found: C, 70·4; H, 4·6; N, 7·9. $C_{11}H_9O_2N$ requires C, 70·6; H, 4·8; N, 7·5%). The 2 : 4-*dinitrophenylhydrazone*, prepared by shaking a solution of the aldehyde in ether with one of the hydrazine in 2N-hydrochloric acid, crystallised from benzene in microscopic, orange-yellow needles, m. p. 224° (Found: C, 56·0; H, 3·5; N, 19·9. $C_{17}H_{13}O_5N_5$ requires C, 55·6; H, 3·5; N, 19·1%).

(ii) A suspension of stannous chloride (20 g.; anhydrous) in ether (100 c.c.) was saturated with hydrogen chloride. 4-Cyano-2-benzyloxazole (10 g.) was melted, diluted with a little ether, and added quickly to the mixture with vigorous stirring, which was continued until the lower layer became too viscous (about 30 minutes). More hydrogen chloride was passed in, and the whole kept overnight. The upper layer was decanted, and the remaining viscous gum added gradually to an excess of aqueous sodium hydrogen carbonate. The crude aldehyde (8·85 g.) was recovered by ether extraction, using a centrifuge to separate the layers; it crystallised on removal of the ether. A portion, recrystallised from ether and sublimed at 60°/0·05 mm., had m. p. 71° (Found: C, 70·8; H, 4·9%).

2-*Phenyloxazole-4-aldehyde*.—2-Phenyloxazole-4-carboxylic acid (10 g.) was boiled gently under reflux with an excess of thionyl chloride for two hours. The residue after removal of thionyl chloride was recrystallised from light petroleum (b. p. 60—80°). The *acid chloride* formed colourless plates (9 g.), m. p. 108—109° (Found: C, 57·8; H, 3·1. $C_{10}H_6O_2NCl$ requires C, 58·0; H, 2·9%). This chloride (5 g.) in xylene (25 c.c.) with palladised barium sulphate (2 g.) and thiourea (10 mg.) was boiled under reflux with occasional shaking while hydrogen was passed in. After 10 hours, 75% of the theoretical quantity of hydrogen chloride had been evolved. The catalyst was removed and washed with ether; the filtrate was shaken with sodium hydrogen sulphite solution (40 c.c.; saturated). After 30 minutes at 0° the crystalline adduct was collected, dissolved in a minimum of water, and decomposed by addition of sodium carbonate. The precipitated 2-*phenyloxazole-4-aldehyde* was recrystallised from light petroleum (b. p. 60—80°), giving 3 g., m. p. 94° (Found: C, 68·9; H, 4·3; N, 8·3. $C_{10}H_7O_2N$ requires C, 68·3; H, 4·0; N, 8·0%). The orange 2 : 4-*dinitrophenylhydrazone* had m. p. 209° after recrystallisation from ethanol (Found: C, 54·4; H, 3·5; N, 19·2. $C_{16}H_{11}O_5N_5$ requires C, 54·4; H, 3·1; N, 19·8%). The aldehyde, in a little alcohol, was treated with a slight excess of aniline; after 6 hours water was added and the *anil* collected; recrystallisation from alcohol gave yellowish prisms, m. p. 123° (Found: C, 77·3; H, 5·4; N, 11·6. $C_{16}H_{12}ON_2$ requires C, 77·4; H, 4·8; N, 11·3%).

Phenylacetamidomalondialdehyde (I or IX; R = CH_2Ph).—2-Benzyloxazole-4-aldehyde (2·93 g.) was boiled with N-sodium hydroxide (50 c.c.) for 10 minutes, clarified with charcoal, cooled, and treated with N-hydrochloric acid (50 c.c.). The *dialdehyde* (2·35 g.) was collected; m. p. 108°. A further small amount could be recovered from the filtrate by ether extraction.

The crude product from the Stephen reduction of 4-cyano-2-benzyloxazole (above) was hydrolysed in similar fashion to give the same dialdehyde (5·6 g. from 8·85 g.). A by-product of this process was 2-*benzyloxazole-4-carboxyamide* which separated on cooling the alkaline liquor and had m. p. 168—169° after crystallisation from alcohol (Found: N, 13·6. $C_{11}H_{10}O_2N_2$ requires N, 13·9%); on alkaline hydrolysis it gave ammonia and 2-benzyloxazole-4-carboxylic acid.

The dialdehyde crystallised from light petroleum in long needles, m. p. 108° (Found: C, 64·5; H, 5·6; N, 6·6; equiv., 205. $C_{11}H_{11}O_3N$ requires C, 64·4; H, 5·4; N, 6·8%; equiv., 205). The equivalent weight was determined by titration with standard barium hydroxide (phenolphthalein). The substance was easily soluble in aqueous sodium hydrogen carbonate and, to some extent, in aqueous sodium acetate. It gave a purple colour with ferric chloride. The ultraviolet-light absorption curve (substance dissolved in 0·02N-sodium hydroxide) showed a maximum at 2670 A. (log ε 4·41) and a minimum at 2350 A. (log ε 3·45). When 50% aqueous methanol was the solvent, the maximum was at the same wave-length; when this solution was made 0·04N. with respect to hydrochloric acid, absorption in this region became weaker and less selective ($\lambda_{max.}$ 2480 A., log ε 4·08).

The *mono*-2 : 4-*dinitrophenylhydrazone* separated from ethyl acetate in minute, brick-red, torpedo-shaped crystals, m. p. 187—190° (dependent on rate of heating) (Found: C, 53·2; H, 4·0; N, 17·7. $C_{17}H_{13}O_6N_5$ requires C, 53·0; H, 3·9; N, 18·2%). An aqueous solution of the dialdehyde was treated with aniline sulphate; next day the precipitate was recrystallised from ethanol giving *β-anilino-α-phenylacetamidoacraldehyde* (X; R = CH_2Ph; R′ = Ph), yellowish prisms, m. p. 235—237° (Found: C, 73·3; H, 5·9; N, 10·0. $C_{17}H_{16}O_2N_2$ requires C, 72·9; H, 5·7; N, 10·0%), which gave a red coloration with ferric chloride in ethanol.

Benzamidomalondialdehyde (I or IX; R = Ph).—2-Phenyloxazole-4-aldehyde (2 g.) was boiled with 2N-sodium hydroxide (20 c.c.) until dissolution was complete (2 minutes). After cooling and acidification of the mixture, the product was collected and recrystallised from light petroleum (b. p. 60—80°). The dialdehyde (2 g.) formed colourless prisms, m. p. 76—77° (Found: C, 62·6; H, 4·9; N, 7·3. $C_{10}H_9O_3N$ requires 3, 62·8; H, 4·7; N, 7·3%). It was acidic, gave a blood-red colour with ferric chloride, and strongly reduced Tollens's reagent. On addition of benzylamine to the dialdehyde (0·5 g.) in ether (20 c.c.), a *benzylamine* salt separated; m. p. 136—137°, after recrystallisation from ethanol (Found: C, 68·5; H, 6·2. $C_{10}H_9O_3N,C_7H_9N$ requires C, 68·4; H, 6·0%). This was melted in a vacuum over phosphoric anhydride. When frothing ceased the residue was recrystallised from ethanol to give *β-benzylamino-α-benzamidoacraldehyde* (X; R = Ph, R′ = CH_2Ph) as colourless plates, m. p. 61—62° (Found: C, 72·4; H, 5·9; N, 10·6. $C_{17}H_{16}O_2N_2$ requires C, 72·9; H, 5·7; N, 10·0%), which gave a red colour with ferric chloride. Aniline sulphate was added to a solution of the dialdehyde (0·5 g.) in water (200 c.c.); after 12 hours the yellow solid was recrystallised from ethanol; *β-anilino-α-benzamidoacraldehyde* (X; R = R′ = Ph) formed needles (0·54 g.), m. p. 152—153°; it gave an almost

black dinitrophenylhydrazone, but no colour with alcoholic ferric chloride (Found: C, 71·9; H, 5·4. $C_{16}H_{14}O_2N_2$ requires C, 72·2; H, 5·2%).

Condensation of Phenylacetamidomalondialdehyde with Penicillamine and Penicillamine Methyl Ester.—A series of experiments on the condensation of phenylacetamidomalondialdehyde with varying amounts of penicillamine or its methyl ester gave amorphous products, formed by the condensation of one molecule of the dialdehyde with two molecules of penicillamine or its ester. In condensations involving the use of less than two molecular proportions of penicillamine or its ester, unchanged dialdehyde has been recovered from the product of the reaction. All attempts to obtain pure crystalline substances from the product failed. The following is a description of a typical experiment. To a suspension of phenylacetamidomalondialdehyde (51 mg.) in *iso*propyl ether (1 c.c.) and ethanol (0·5 c.c.) was added a solution of D-penicillamine hydrochloride (88 mg.; slightly less than 2 mols.) in water (0·5 c.c.). Sodium acetate crystals (66 mg.) were added, and the mixture was shaken at room temperature. After 2 hours the reaction mixture still gave a blue coloration with ferric chloride, but there was no coloration at the end of 5 hours. The mixture was evaporated to dryness *in vacuo* at room temperature, and the residue was triturated with excess of a saturated solution of sodium hydrogen carbonate. The solution was filtered, the filtrate extracted with ether, and the extract, found to contain only 3 mg. of dissolved solid material, was discarded. On acidification to pH 2 with N-hydrochloric acid, a white precipitate (40 mg.) was formed. This was collected, and a further quantity (22 mg.) of the white substance was obtained by extracting the filtrate with ether. When heated, this amorphous product started to soften at about 120° and gradually melted, with decomposition and evolution of gas, at 140—150°. Attempts to obtain a pure crystalline substance from this product by manipulation with solvents were unsuccessful (Found: C, 54·2; H, 6·4; N, 8·6. $C_{21}H_{29}O_5N_3S_2$ requires C, 54·0; H, 6·2; N, 9·0%).

Another specimen of the condensation *product*, prepared in a similar way, had equiv. 235·6. A dicarboxylic acid, $C_{21}H_{29}O_6N_3S_2$, requires equiv. 233·5.

D-Penicillamine methyl ester also yielded a bisthiazolidine derivative.

Many further experiments were made, but, for the reason already mentioned, need not be described at this stage.

Hexamidomalondialdehyde (I or IX; R = *n*-C_5H_{11}).—2-Amyloxazole-4-aldehyde was hydrolysed with N-sodium hydroxide as described in the other examples. The *dialdehyde* separated from light petroleum (b. p. 60—80°) in colourless needles, m. p. 66—67° (Found: C, 58·2; H, 8·1; N, 7·9. $C_9H_{15}O_3N$ requires C, 58·4; H, 8·1; N, 7·6%). It dissolved in aqueous sodium hydrogen carbonate and gave a blood-red colour with ferric chloride.

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331. *Some Physical Properties of Tetranitromethane.*

By A. J. C. NICHOLSON.

It has been shown that the method normally used for the preparation of tetranitromethane yields a very impure product. New values are given for the freezing point, refractive index, density, and vapour pressure of tetranitromethane. The absorption spectrum of the vapour has been photographed.

THE generally accepted view of the structure of tetranitromethane has been that it is a symmetrical molecule consisting of four true "nitro-"groups arranged tetrahedrally around a central carbon atom. From time to time it has been argued that one of these groups is different from the other three. Several studies of physical properties of tetranitromethane have aimed at deciding between these possibilities. However, there is no general agreement on the physical constants of the pure substance. Sometimes the method of preparation was not indicated. Where methods and constants are given, the freezing points and refractive indices variously quoted differ widely. The preparation and purification of tetranitromethane are discussed below, and new values are given for some physical constants which are compared with those obtained by previous workers.

EXPERIMENTAL.

Chattaway's convenient preparation (*J.*, 1910, **97**, 2099) gave a product so impure (f. p. 10·5—13·0°) that a prohibitive number of fractional distillations were required to yield reasonable purity. Liang's modification of Chattaway's method (*Org. Syntheses*, 1941, **21**, 105) gives a much purer product (f. p. 13·0—13·5°), the essential purifying step being steam-distillation. Since the residual solution becomes bright yellow during the distillation, the impurity removed by this process may be trinitromethane. After steam-distillation the tetranitromethane was dried (Na_2SO_4) and fractionated, at low pressures, in a micro-still (cf. Schrader and Ritzer, *Ind. Eng. Chem., Anal. Edn.*, 1939, **11**, 54). The column was packed with Fenske rings and reflux was obtained by means of a cold pencil. At 28 mm. a small fraction (probably water) distilled at *ca.* 30°; and the remainder, b. p. *ca.* 42°, was divided into five fractions. The distillation was then repeated using fractions 3, 4, and 5, and although in the

second distillation all the fractions distilled within 0·3° fractions 1, 2, and 6 were again discarded. The tetranitromethane from this distillation was then distilled into an all-glass apparatus from room temperature to liquid-air temperature across phosphoric oxide. The phosphoric oxide tube was then sealed off and the liquid finally divided into five fractions by vacuum-distillation to liquid-air temperatures. Fractions 1 and 5 were discarded; fraction 3 was used on the apparatus, and small fractions 2 and 4 were used for measurements of physical properties. The values for fractions 2 and 4 were always identical. Four separate batches of tetranitromethane were submitted to this complete purification and all finally gave the same m. p. and refractive index. The liquid obtained always had a faint yellow colour when viewed in fair thickness. Some workers have claimed that the pure substance is colourless.

Freezing Point.—F. p.s were measured by plotting cooling curves for the liquid. The thermometer was read to 0·1° and was calibrated to this accuracy against a N.P.L.-standardised thermometer.

Refractive Index.—The refractive Index was measured with an Abbé refractometer (Hilger), read to 0·0002 unit and compensated to give the refractive index for the Na_D line. Temperatures were ±0·2°, and the instrument was calibrated with a glass sample supplied by the manufacturers and with distilled water.

Density.—Densities were measured with a pyknometer calibrated with distilled water. Only 1-ml. samples of the purest tetranitromethane were available, so the densities are accurate only to about 1 part in 5000. Temperatures were controlled to 0·1°.

Vapour Pressure.—Some measurements of vapour pressure were made at room temperatures in an all-glass system with a Bourdon spoon gauge. Tetranitromethane vapour attacks tap grease so rapidly that reliable measurements cannot be made even in the presence of only one greased tap. These vapour-pressure measurements, together with the b. p.s found around 40° during various fractional distillations, gave a good straight line on a conventional plot of the logarithm of the vapour pressure against the reciprocal of the absolute temperature. The straight line could be represented by $\log_{10} p(\text{mm. Hg.}) = 8{\cdot}63 - 2260/T(°\text{K.})$, and extrapolates to give reasonable agreement with Menzies's vapour-pressure measurements (*J. Amer. Chem. Soc.*, 1919, **41**, 1336) at higher temperatures. From this equation the latent heat of vaporisation was calculated as 10·3 kcals./g.-mol. Smoothed values for the vapour pressure are:

°c.	0	13·8	20	30	40
V. p., mm. (Hg)	1·9	5·7	8·4	14·9	25·8

Absorption Spectrum.—The absorption spectrum of tetranitromethane vapour was photographed using a Hilger spectrograph (medium quartz type E3). With a path length of 1 metre and a pressure of 7 mm., absorption starts at 3050 A., increases to a max. at 2750 A., decreases to a min. at 2500 A., and becomes continuous below 2400 A. No trace of a banded structure was observed.

Explosion Risk.—Because of the reputed explosion risk (Liang, *loc. cit.*; Macbeth, *Ber.*, 1913, **46**, 2537), tetranitromethane was handled only in small quantities. The material used in the present work did not detonate when hammered between steel surfaces. If a small quantity of benzene was added, this treatment caused a violent detonation. It is unwise to dogmatise over questions of explosive sensitivity but it seems probable that pure tetranitromethane is a comparatively insensitive explosive as far as impact shock is concerned, but that it becomes very sensitive when contaminated with aromatic substances or alkali (cf. Bowden, Mulcahy, Vines, and Yoffe, *Proc. Roy. Soc.*, 1947, **188**, *A*, 291). Steam-distillation seems a safe process. No change in properties occurred when tetranitromethane was kept in glass vessels at room temperature for several weeks. At room temperatures the vapour attacks tap grease, rapidly giving off gaseous decomposition products, and also attacks mercury. Thus "vacuum-stability tests" of the type described by Farmer (*J.*, 1920, **117**, 1432), carried out at high temperatures in the presence of both mercury and grease, greatly exaggerate the "instability" of the liquid.

The table below collects values of physical constants given by us and others. Other authors quote a wide range of freezing points, none, however, higher than 13·5°. For ease of comparison some corrections have been applied as set out in the footnotes below the table.

		Density (g./ml.).			n_D.	
Ref.	F. p., °c.	15°.	20°.	25°.	20°.	25°.
1	—	(*a*) 1·6446	—	1·6336	1·4382	1·4359
		(*b*) 1·648	—	1·631	—	—
2	13·75	1·650	—	—	—	—
3	13·0	1·646	—	—	1·4383	—
4	12·5—13·5	—	1·638	1·629	—	—
5	11·5—12·5	—	—	—	—	—
6	—	—	—	1·62294	—	1·43822
7	13·8	1·649	—	1·630	1·4384	1·4358

[1] von Auwers and Ottens, *Ber.*, 1924, **57**, 447, and von Auwers and Herres, *Ber.*, 1929, **62**, 2287. The refractive indices are interpolated from their figures for the hydrogen lines at different temperatures. The density values (*a*) and (*b*) are calculated from their values at 16·9° and 21·2°, the author's value for the temperature coefficient of density being used for (*b*).

[2] Orton and McKie, *J.*, 1920, **117**, 283. Density calculated from their specific gravity at 15·8° using the author's value for the temperature coefficient.

[3] Pictet and Genequand, *Ber.*, 1903, **36**, 2225. Calculated using the author's temperature coefficients.

[4] Hammick and Young, *J.*, 1934, 32; 1936, 1463.

[5] Coop and Sutton, *J.*, 1938, 1269. F. p. measured before one fractional distillation.

[6] Lewis and Smyth, *J. Amer. Chem. Soc.*, 1939, **61**, 3067.

[7] This work.

DISCUSSION.

The tetranitromethane used in the present work was more carefully fractionated than that used by other workers and had the highest recorded freezing point; its vapour pressure, refractive index, and density agree well with the most concordant recorded values. The values here recorded are claimed as more reliable than those previously accepted.

Mark and Noethling (*Z. Kryst.*, 1927, **65**, 435) suggested, from a Debye–Scherrer study of crystalline tetranitromethane, that one of the nitro-groups has a nitrite structure. This view receives no support from the absorption spectrum of the vapour; for the spectrum is almost identical with that described by Hirschlaff and Norrish (*J.*, 1936, 1580) and Thompson and Purkis (*Trans. Faraday Soc.*, 1936, **32**, 674) for nitromethane and shows no trace of the banded structure found by the latter authors for the alkyl nitrites. Coop and Sutton (*loc. cit.*) and Lewis and Smyth (*loc. cit.*) measured the dipole moment of tetranitromethane and obtained values different from zero, although in both papers it was considered that the difference could be accounted within the experimental error of their methods; their tetranitromethane was prepared by Chattaway's method and purified by one distillation; since these materials were far from pure (cf. table), the impurity might suffice to explain the dipole-moment results. Most of the more recent physicochemical work of Médard (*J. Chim. physique*, 1935, **32**, 136) and Mathieu and Massignon (*Compt. rend.*, 1940, **211**, 323) on infra-red and Raman spectra, and of Stosick (*J. Amer. Chem. Soc.*, 1939, **61**, 1127) on electron diffraction, favours the symmetrical structure with four true " nitro-"groups.

My thanks are due to Professor R. G. W. Norrish, F.R.S., for much advice and encouragement and to the Royal Commissioners for the Exhibition of 1851 for the award of an Overseas Scholarship.

DEPARTMENT OF PHYSICAL CHEMISTRY, CAMBRIDGE. [*Received, January 4th*, 1949.]

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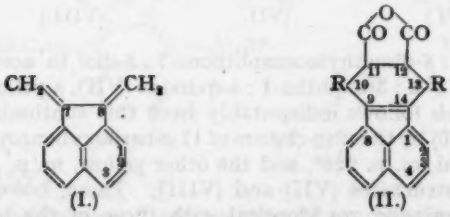
332. *Syntheses of Fluoranthene and its Derivatives from 7 : 8-Dialkylacenaphthene-7 : 8-diols.*

By NEIL CAMPBELL and R. S. GOW.

7 : 8-Dialkylacenaphthene-7 : 8-diols, when heated with dienophiles in dehydrating solvents, condense readily to give fluoranthene derivatives which on appropriate treatment yield substituted fluoranthenes. In this way fluoranthene, 4-bromofluoranthene, 10 : 13-*dimethylfluoranthene*, *fluorantheno*(11′ : 12′-2 : 3)-p-*benzoquinone*, *fluorantheno*(11′ : 12′-2 : 3)*naphtha*-1 : 4-*quinone*, *naphtho*(2′ : 3′-11 : 12)*fluoranthene*, 11 : 12-*di*-p-*toluoylfluoranthene*, and 1′ : 4′-*di*-p-*tolyl*-11 : 12-*benzfluoranthene* have been synthesised. Certain statements in the literature have been corrected.

ACENAPHTHENE-7 : 8-QUINONE reacts easily with Grignard reagents to give 7 : 8-dialkylacenaphthene-7 : 8-diols (Maxim, *Bull. Soc. chim.*, 1928, **43**, 769; 1929, **45**, 1137). Theoretically a mixture of *cis*- and *trans*-forms may result, but Maxim found that this holds only for the dimethylglycol; the diethyl and dibenzyl compounds, for instance, were obtained in one form only, and this is in agreement with the results of Bachmann and Chu (*J. Amer. Chem. Soc.*, 1936, **58**, 1118) on the formation of 7 : 8-diarylacenaphthene-7 : 8-diols.

Criegee *et al.* (*Annalen*, 1933, **507**, 176) improved Maxim's method for separating the two 7 : 8-dimethylacenaphthene-7 : 8-diols and proved the isomers, m. p. 187—189° and 182—183°, to be the *cis*- and *trans*-compounds respectively. On treating the former substance with glacial acetic acid, Maxim (*loc. cit.*) isolated a hydrocarbon which he claimed to be 7 : 8-dimethyleneacenaphthene (I). This substance has a high m. p. and is almost insoluble in organic solvents,



in contrast to the other 7 : 8-dialkylideneacenaphthenes, and is probably a polymer of dimethyleneacenaphthene. It was, therefore, decided to generate the diene by dehydrating the glycol

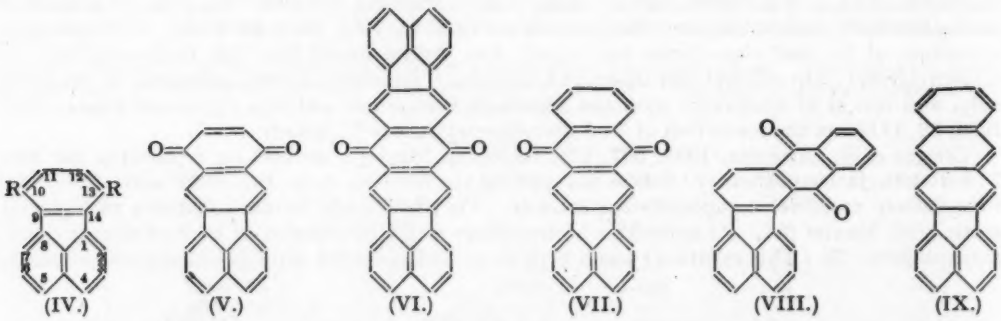
and simultaneously to condense it with a dienophile. This proved to be successful and *trans*-7 : 8-dimethylacenaphthene-7 : 8-diol, when heated under reflux in acetic anhydride with maleic anhydride for 15 minutes, smoothly yielded 10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic anhydride* (II; R = H) which was dehydrogenated by chloranil to *fluoranthene*-11 : 12-*dicarboxylic anhydride* (III; R = H). Decarboxylation was difficult, but, when the tetrahydro-compound (II) was heated with alkaline potassium ferricyanide (cf. Wagner-Jauregg, *Annalen*, 1931, **491**, 1), dehydrogenation and decarboxylation proceeded simultaneously to give a 50% yield of fluoranthene (IV; R = H).

Use of *cis*-7: 8-dimethylacenaphthene-7 : 8-diol in the diene reaction gave only a red polymer.

Acenaphthenequinone and ethylmagnesium iodide gave only one isomer of 7 : 8-diethylacenaphthene-7 : 8-diol, presumably the *trans*-form as it afforded with maleic anhydride in acetic anhydride a 33% yield of 10 : 13-*dimethyl*-10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic anhydride* (II; R = Me), which on dehydrogenation by chloranil gave 10 : 13-*dimethylfluoranthene*-11 : 12-*dicarboxylic anhydride* (III; R = Me). Decarboxylation yielded 10 : 13-*dimethylfluoranthene* (IV; R = Me).

3-Bromoacenaphthenequinone with methylmagnesium iodide gave 3-*bromo*-7 : 8-*dimethylacenaphthene*-7 : 8-*diol*, probably a mixture of the *cis*- and *trans*-isomers since it melted over a range of 10° and did not react satisfactorily with maleic anhydride in acetic anhydride. Repeated crystallisation from benzene gave, presumably, the *trans*-form, m. p. 167—168°, which reacted easily with maleic anhydride in acetic anhydride to give 4-*bromo*-10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic anhydride*, which was dehydrogenated by chloranil to 4-*bromofluoranthene*-11 : 12-*dicarboxylic anhydride*. Decarboxylation was troublesome and the end product, 4-bromofluoranthene, was best obtained by treatment of the tetrahydro-anhydride with alkaline potassium ferricyanide. 4-Bromofluoroanthene thus synthesised was identical with the main substitution product of fluoranthene and so confirms the orientation studies of von Braun and Manz (*Annalen*, 1931, **488**, 111).

trans-7 : 8-Dimethylacenaphthene-7 : 8-diol with *p*-benzoquinone gave *fluorantheno*-(11′ : 12′-2 : 3)-p-*benzoquinone* (V), the intermediate tetrahydro-derivative apparently being dehydrogenated by excess of benzoquinone. The dienophilic character of the product is shown by its ability to react with a second molecule of the *trans*-diol to give *difluorantheno*(11′ : 12′-2 : 3)(11″ : 12″-5 : 6)-p-*benzoquinone* (VI), again no tetrahydro-intermediate being isolated. All attempts, including zinc-dust distillation and Clar's zinc–zinc chloride melt, failed to reduce the quinone (V) to the corresponding hydrocarbon. The quinone, it may be noted, differs from that obtained by Moureu *et al.* (*Bull. Soc. chim.*, 1948, 99) by the oxidation of 11 : 12-benzfluoranthene, and thus confirms the constitution assigned without proof by Moureu to his product.



(IV.) (V.) (VI.) (VII.) (VIII.) (IX.)

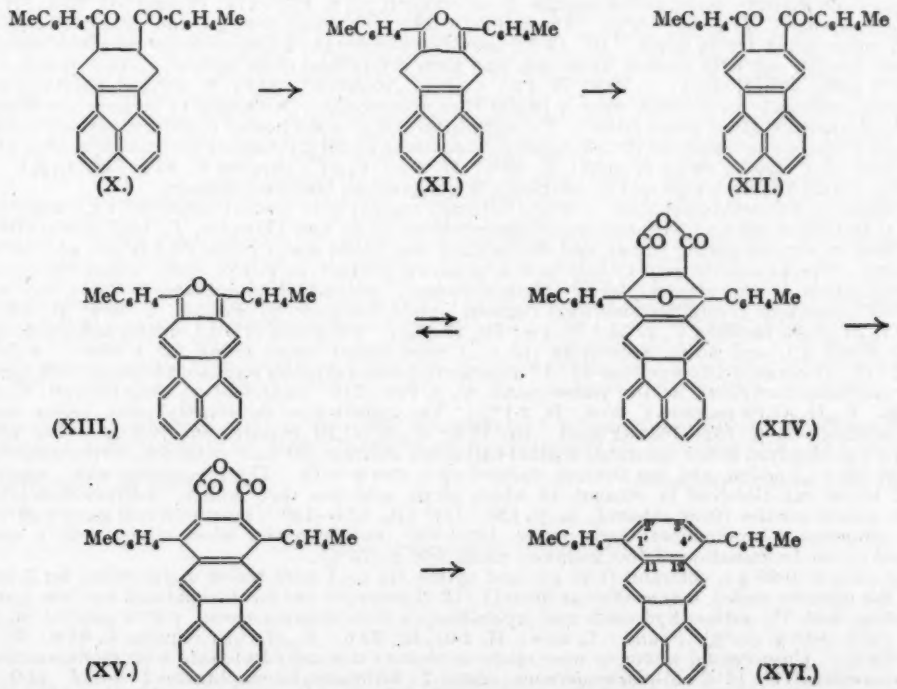
With 1 : 4-naphthaquinone *trans*-7 : 8-dimethylacenaphthene-7 : 8-diol in acetic anhydride gave a good yield of fluorantheno(11′ : 12′-2 : 3)naphtha-1 : 4-quinone (VII), a yellow compound, m. p. > 310°, the constitution of which follows indisputably from this synthesis. Now, von Braun and Manz (*Annalen*, 1932, **496**, 170) by the ring-closure of 11-*o*-carboxybenzoylfluoranthene obtained two isomeric quinones, one red, m. p. 228°, and the other yellow, m. p. 332—333°, to which were assigned respectively the structures (VII) and (VIII). These, however, must be reversed since the properties of our quinone are identical with those of the higher-melting quinone. The conclusions of von Braun and Manz were based on oxidation studies which are incomplete and unreliable since, of the three expected oxidation products from the two quinones,

they isolated only one in anything approaching the pure condition and this they failed to identify by mixed-m.p. determination with an authentic sample.

The quinone (VII) was converted into naphtho(2′ : 3′-11 : 12)fluoranthene (IX) by fusion with zinc and zinc chloride (Clar, *Ber.*, 1939, **72**, 1645), m. p. > 310°. von Braun and Manz's compound (*loc. cit.*), obtained by zinc-dust distillation and by implication erroneously regarded as the 10 : 11-fluoranthene derivative, had m. p. 290—291° and must have been impure.

Clar (" Aromatische Kohlenwasserstoffe," p. 18) correlated the oxidation–reduction potential of quinones with their ability to give vats, and it is therefore of interest to compare the quinones (V), (VII), and (VI). (V) gave a wine-red vat, discharged overnight by alkaline dithionite, zinc dust and ammonia, or sodium hydroxide; (VII) gave, only with dithionite, a vat which was stable for a few hours; and (VI), only with dithionite, gave a pale blue vat, stable for a few seconds. The stability of the vats is clearly in the order, (V) > (VII) > (VI).

trans-Di-*p*-toluoylethylene and the dimethylacenaphthenediol in acetic anhydride yielded the *iso*benzfuran derivative (XI), presumably through the intermediate (X) which enolises and then loses a molecule of water. On hydrolysis with 50% sulphuric acid, (XI) underwent both ring-fission and dehydrogenation to give 11 : 12-*di*-p-*toluoylfluoranthene* (XII), the full aromaticity of which was shown by its failure to give tetrachloroquinol when heated in xylene with chloranil. (XI) was dehydrogenated to 2 : 5-*di*-p-*tolylfluorantheno*(11′ : 12′-3 : 4)*furan* (XIII), the orange colour of which is due to its conjugation. With 50% sulphuric acid, (XIII) gave (XII), hydrolysis again being accompanied by dehydrogenation. The constitution of the furan derivative (XIII) was proved by its combination with maleic anhydride to give the *adduct* (XIV). This addition is characterised by its occurrence in a few seconds at room temperature and the disappearance of all colour; when the mixture is warmed, the yellow colour and characteristic yellowish-orange fluorescence of the furan (XIII) reappear, thus showing the dissociation of the adduct into its generators. Such dissociation of Diels–Alder adducts is not unknown (*e.g.*, Bachmann, *J. Amer. Chem. Soc.*, 1938, **60**, 481) and has been observed with *iso*benzfuran derivatives by Barnett (*J.*, 1935, 1326). The adduct (XIV), on treatment with hydrobromic acid, lost water to give the *substance* (XV) which yielded on decarboxylation 1′ : 4′-*di*-p-*tolyl*-11 : 12-*benzfluoranthene* (XVI).



The synthetic value of the above reactions depends on the nature of both the diol and the dienophile. For example, maleic anhydride reacts in acetic anhydride with the dimethyl-diol in 15 minutes giving a 70% yield of the adduct, and with the diethyl diol in 5 hours to give a

33% yield, but with the dibenzyl diol no reaction occurred. Highly reactive dienophiles such as maleic anhydride or benzoquinone are required, since other substances such as vinyl cyanide and crotonic acid give only polymeric products.

Experimental.

All analyses were done by Drs. Weiler and Strauss, Oxford.

Synthesis of Fluoranthene.—trans-7 : 8-Dimethylacenaphthene-7 : 8-diol (1·0 g.), maleic anhydride (4 g.), and acetic anhydride (30 c.c.) were heated under reflux for 15 minutes. The cooled solution deposited 10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic anhydride*, yellow elongated prisms (from ethanol–acetic acid), m. p. 209—210° (yield, 0·9 g., 70%) (Found: C, 77·6; H, 4·9. $C_{18}H_{12}O_3$ requires C, 78·25; H, 4·4%). The *dimethyl* ester crystallised from ethanol in yellow prisms, m. p. 153° (Found: C, 73·8; H, 5·8. $C_{20}H_{18}O_4$ requires C, 74·5; H, 5·6%). The anhydride (1·15 g.), chloranil (2·05 g.), and sulphur-free xylene (30 c.c.) were boiled under reflux for 24 hours, and the adduct which separated on cooling was purified by trituration with 5% aqueous sodium hydroxide and crystallisation from acetic acid. *Fluoranthene*-11 : 12-*dicarboxylic anhydride* was thus obtained as needles, m. p. 299—300°, with a faint orange fluorescence and a blue fluorescence in acetic acid (yield, 1·0 g., 95%) (Found: C, 79·0; H, 3·1. $C_{18}H_8O_3$ requires C, 79·4; H, 3·0%). The *dimethyl* ester was obtained by boiling the anhydride under reflux with sulphuric acid in methanol for 11 hours and crystallised from ethanol in large plates, m. p. 127—128° (Found: C, 75·5; H, 4·6. $C_{20}H_{14}O_4$ requires C, 75·5; H, 4·4%). The anhydride, when heated with calcium oxide, gave a 50% yield of fluoranthene, identified by its m. p. and mixed m. p. with an authentic coal-tar sample and by the m. p. of the picrate.

The anhydride was not readily decarboxylated, heating with copper and quinoline or with mercuric oxide at 250° in a sealed tube (Dziewonski and Kahl, *Chem. Abs.*, 1935, **29**, 2941) proving unsuccessful and heating with calcium oxide wasteful. With naphthalic anhydride the second method gave 80% of naphthalene. Tetrahydrofluoranthene-11 : 12-dicarboxylic anhydride (0·5 g.) in hot 10% sodium hydroxide (40 c.c.) was added to potassium ferricyanide (4·5 g.) in water (20 c.c.) and heated for 3 hours; the solution deposited fluoranthene in 50% yield.

Synthesis of 10 : 13-*Dimethylfluoranthene.*—7 : 8-Diethylacenaphthene-7 : 8-diol (0·5 g.), maleic anhydride (2 g.), and acetic anhydride (15 c.c.) were boiled under reflux for 5 hours. 10 : 13-*Dimethyl*-10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic anhydride* separated and crystallised from acetic acid in yellow cubic crystals, m. p. approx. 260° depending on the rate of heating (yield, 0·2 g., 33%) (Found: C, 78·5; H, 5·2. $C_{20}H_{16}O_3$ requires C, 78·9; H, 5·3%). The anhydride dissolved in hot sodium hydroxide to give with hydrochloric acid the *acid*, m. p. 218°, which did not crystallise well from ethanol or acetic acid (Found: C, 73·65; H, 5·7. $C_{20}H_{18}O_4$ requires C, 74·5; H, 5·6%). The adduct (0·1 g.), when boiled under reflux for 9 hours with 10 c.c. of methanol containing 1 c.c. of sulphuric acid gave the *dimethyl* ester, yellow needles (from ethanol), m. p. 173—174° (Found: C, 75·3; H, 6·5. $C_{22}H_{22}O_4$ requires C, 75·4; H, 6·3%). The adduct (0·28 g.), chloranil (0·45 g.), and xylene (10 c.c.) were heated under reflux for 24 hours. 10 : 13-*Dimethylfluoranthene*-11 : 12-*dicarboxylic anhydride* separated and was washed out with sodium hydroxide and then crystallised from xylene; yellow plates, m. p. > 300°; yield, 50% (Found: C, 79·8; H, 4·3. $C_{20}H_{12}O_3$ requires C, 80·0; H, 4·0%). Solutions of the anhydride, especially in acetone, show a bright blue fluorescence. Attempts to prepare the dimethyl ester by the method given above failed. The anhydride (0·5 g.) when heated to dull redness with calcium oxide (50 g.) gave a sublimate of 10 : 13-*dimethylfluoranthene* which crystallised from acetic acid in plates, m. p. 210—211° (yield, 25%) (Found: C, 93·6; H, 6·1. $C_{18}H_{14}$ requires C, 93·9; H, 6·1%). The solid has a yellow fluorescence and in solution a bright greenish-blue fluorescence.

Synthesis of 4-*Bromofluoranthene.*—To the Grignard reagent from methyl iodide (11·1 g.), magnesium (1·9 g.), and ether (50 c.c.) 3-bromoacenaphthenequinone (Rule and Thomson, *J.*, 1937, 1761) (10·2 g.) was added in portions during ½ hour, and the mixture was boiled under reflux for 2 hours, and set aside overnight. Decomposition with 4N-sulphuric acid gave a product, m. p. 150—160°, which was obtained by evaporation of the ethereal layer. trans-3-*Bromo*-7 : 8-*dimethylacenaphthene*-7 : 8-*diol* has m. p. 167—168° after four crystallisations from benzene; yield, 5·0 g. (50%) (Found: C, 56·9; H, 4·6; Br, 27·2. $C_{14}H_{13}O_2Br$ requires C, 57·35; H, 4·5; Br, 27·3%). The glycol (1·0 g.), maleic anhydride (5 g.), quinol (0·001 g.), and acetic anhydride (15 c.c.) were boiled under reflux for 1 hour. 4-*Bromo*-10 : 11 : 12 : 13-*tetrahydrofluoranthene*-11 : 12-*dicarboxylic acid anhydride* separated from the cold mixture and crystallised from acetic acid in yellow plates, m. p. 213—216° (yield, 0·51 g., 43%) (Found: C, 60·2; H, 3·45. $C_{18}H_{11}O_3Br$ requires C, 60·9; H, 3·1%). The adduct gave the *dimethyl* ester, yellow needles (from ethanol), m. p. 152—154° (Found: Br, 19·3. $C_{20}H_{17}O_4Br$ requires Br, 19·9%). The adduct (0·40 g.) was dissolved in hot saturated sodium carbonate solution (30 c.c.), potassium ferricyanide (4 g.) in water (20 c.c.) added, and the solution warmed on a steam-bath. The precipitate which separated after 2 hours was dissolved in ethanol, to which picric acid was then added. 4-Bromofluoranthene picrate, yellow needles (from ethanol), m. p. 130—131° (lit., 129—130°), separated and gave with warm dilute ammonia 4-bromofluoranthene, m. p. 103—105°, not depressed when mixed with a sample obtained by the bromination of fluoranthene; yield, 0·06 g. (25%).

The adduct (0·95 g.), chloranil (1·32 g.), and xylene (12 c.c.) were boiled under reflux for 3 hours. When the mixture cooled, 4-*bromofluoranthene*-11 : 12-*dicarboxylic anhydride* separated and was purified by washing with 5% sodium hydroxide and crystallisation from chlorobenzene; yellow needles, m. p. > 310°; yield, 0·40 g. (42%) (Found: C, 61·4; H, 2·0; Br, 22·5. $C_{18}H_7O_3Br$ requires C, 61·6; H, 2·0; Br, 22·75%). Unsuccessful attempts were made to convert this anhydride into 4-bromofluoranthene.

Fluorantheno(11′ : 12′-2 : 3)-p-*benzoquinone.—trans*-7 : 8-Dimethylacenaphthene-7 : 8-diol (4·0 g.), benzoquinone (16 g.), quinol (0·001 g.), and acetic anhydride (25 c.c.) were heated under reflux for 1½ hours and deposited, on cooling, *fluorantheno*(11′ : 12′-2 : 3)-p-*benzoquinone* which was boiled with water and then crystallised from xylene (charcoal): yellow prisms, m. p. 265° (decomp.), depends on rate of heating; yield, 1·7 g. (30%) (Found: C, 84·7; H, 3·6. $C_{22}H_{10}O_2$ requires C, 85·05; H, 3·6%). The

quinone gives a blue colour with concentrated sulphuric acid, and a yellow fluorescence either in the solid state or in solution.

Difluorantheno(11′ : 12′-2 : 3)(11″ : 12″-5 : 6)-p-*benzoquinone*.—A mixture of the above quinone (0·5 g.), *trans*-7 : 8-dimethylacenaphthene-7 : 8-diol (0·25 g.), quinol (0·001 g.), and acetic anhydride (200 c.c.) was heated under reflux for 12 hours. *Difluorantheno*(11′ : 12′-2 : 3)(11″ : 12″-5 : 6)-p-*benzoquinone* slowly formed and crystallised from *o*-dichlorobenzene in brown prisms, m. p. > 300° (yield, 0·1 g., 8%) (Found : C, 88·2; H, 3·5. $C_{34}H_{16}O_2$ requires C, 89·5; H, 3·5%). The quinone gives a blue colour with concentrated sulphuric acid.

Naphtho(2′ : 3′-11 : 12)*fluoranthene*.—*trans*-7 : 8-Dimethylacenaphthene-7 : 8-diol (2·0 g.), 1 : 4-naphthaquinone (6·0 g.), quinol (0·001 g.), and acetic anhydride (35 c.c.) were boiled under reflux for 1½ hours, and the precipitated fluorantheno(11′ : 12′-2 : 3)naphtha-1 : 4-quinone was washed with ether and crystallised from *o*-dichlorobenzene; yellow needles, m. p. > 310°; yield, 1·80 g. (54%) (Found : C, 86·1; H, 4·1. Calc. for $C_{34}H_{18}O_2$: C, 86·7; H, 3·65%). This gives a blue colour with concentrated sulphuric acid. This quinone (0·2 g.), zinc dust (0·2 g.), sodium chloride (0·2 g.), and zinc chloride (1·0 g.) were thoroughly mixed, a drop of water was added, and the mixture fused at 210°. The temperature was raised during 6 minutes to 290°, and the product was then cooled and boiled with dilute hydrochloric acid. The precipitate was chromatographed in benzene on a column of alumina (8 × 1 in.) and a yellow band was eluted with benzene to give naphtho(2′ : 3′-11 : 12)fluoranthene, yellow crystals, m. p. > 310° (yield, 0·02 g., 10%) (Found : C, 95·25; H, 5·2. Calc. for $C_{24}H_{14}$: C, 95·3; H, 4·7%). The hydrocarbon shows a yellowish-blue fluorescence in benzene.

11 : 12-*Di*-p-*toluoylfluoranthene*.—*trans*-7 : 8-Dimethylacenaphthene-7 : 8-diol (4·3 g.), *trans*-di-*p*-toluoylethylene (5·3 g.), quinol (0·001 g.), and acetic anhydride (40 c.c.) were heated under reflux for 1½ hours. The *furan* derivative (XI) separated and crystallised from chlorobenzene in yellow prisms, m. p. 301—302° (yield, 2·6 g., 30%) (Found : C, 90·3; H, 5·5. $C_{32}H_{24}O$ requires C, 90·5; H, 5·7%). The furan derivative (0·1 g.) was boiled under reflux in 50% aqueous sulphuric acid (6 c.c.) and acetic acid (50 c.c.) for 2 hours. The solution on cooling deposited 11 : 12-*di*-p-*toluoylfluoranthene*, which separated from acetic acid in needles, m. p. 260—261°; yield, 0·085 g. (84%) (Found : C, 87·2; H, 5·3. $C_{32}H_{22}O_2$ requires C, 87·6; H, 5·1%). The furan derivative (XI) (1·26 g.), chloranil (0·75 g.), and sulphur-free xylene (130 c.c.) were boiled under reflux for 16 hours. When the mixture cooled, 2 : 5-*di*-p-*tolylfluorantheno*(11′ : 12′-3 : 4)*furan* separated and crystallised from chlorobenzene in orange-red prisms, m. p. 289—290° (yield, 0·90 g., 71%) (Found : C, 90·7; H, 5·6. $C_{32}H_{22}O$ requires C, 90·95; H, 5·3%). Unlike the dihydro-compound it is soluble in benzene in which it displays a magnificent yellowish-orange fluorescence. The furan (0·1 g.) was heated under reflux for 2 hours in 50% sulphuric acid (5 c.c.) and acetic acid (35 c.c.). 11 : 12-Di-*p*-toluoylfluoranthene separated and after crystallisation from acetic acid had m. p. 260—262° (yield, 0·05 g., 49%), and showed no m. p. depression when mixed with a sample obtained as above.

1′ : 4′-*Di*-p-*tolyl*-11 : 12-*benzfluoranthene*.—When molar quantities of maleic anhydride and fluoranthenofuran were dissolved in benzene, the orange colour and orange-yellow fluorescence of the furan disappeared and prisms of the *adduct* (XIV) separated. When, however, the solution was warmed, the colour and fluorescence reappeared. Displacement of the equilibrium to the adduct side was effected by cooling or by adding excess of maleic anhydride to the hot solution. The adduct was best prepared as follows. Maleic anhydride (0·6 g.) was added to the furan (0·1 g.) suspended in ethanol (8 c.c.), and the mixture heated under reflux. After 20 minutes separation of the adduct was complete. The adduct was washed with ether, but could not be crystallised from acetic acid since dissociation occurred; m. p. 220—264° (varies with rate of heating) (Found : C, 83·3; H, 4·75. $C_{36}H_{24}O_4$ requires C, 83·05; H, 4·65%). (XIV) (0·4 g.), maleic anhydride (0·4 g.), and ethanol (50 c.c.) were heated under reflux, and hydrogen bromide passed through the mixture. Dissolution soon occurred and a faint purple fluorescence appeared. After 1 hour the solution was evaporated to about 15 c.c. and on cooling deposited 1′ : 4′-*di*-p-*tolyl*-11 : 12-*benzfluoranthene*-2′ : 3′-*dicarboxylic anhydride*, which separated from chlorobenzene in yellow needles, showing in solution, and especially in acetone, a bright blue fluorescence; m. p. > 310°; yield, 0·2 g. (40%) (Found : C, 85·2; H, 4·5. $C_{36}H_{22}O_3$ requires C, 86·05; H, 4·4%). The yellow sublimate obtained by heating the anhydride (1·0 g.) at red heat with calcium oxide (50 g.) was dissolved in toluene (100 c.c.) and chromatographed on alumina (12 × 1 in.). Development with toluene gave a yellow zone which was eluted with the same solvent. Evaporation afforded 1′ : 4′-*di*-p-*tolyl*-11 : 12-*benzfluoranthene*, which crystallised from acetic acid in yellow needles with a blue fluorescence in toluene; m. p. 289°; yield, 0·10 g. (12%) (Found : C, 93·6; H, 5·7. $C_{34}H_{24}$ requires C, 94·4; H, 5·6%).

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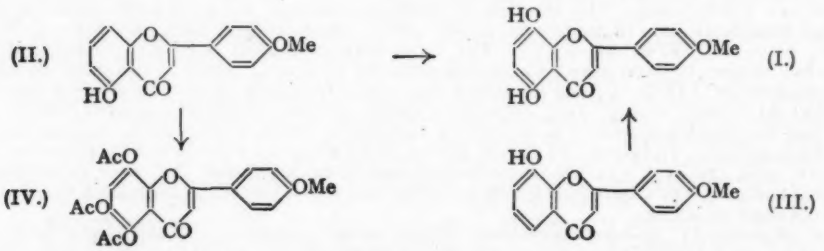
UNIVERSITY OF EDINBURGH. [*Received, January 19th,* 1949.]

333. *The Structure of Ginkgetin. Part II. New Syntheses of 5 : 8-Dihydroxy-4′-methoxyflavone.*

By Wilson Baker, G. F. Flemons, and R. Winter.

Pure 5 : 8-dihydroxy-4′-methoxyflavone (I) has been synthesised by the oxidation of both 5- and 8-*hydroxy-4′-methoxyflavone* with alkaline potassium persulphate.

Ginkgetin was supposed by Furukawa (*Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1932, **19**, 27; 1933, **21**, 278) to be 5 : 8-dihydroxy-4′-methoxyflavone (I), and the synthesis of this compound from 2 : 3-dihydroxy-6-methoxyacetophenone by the Allan–Robinson fusion method was described in Part I of this series (*J.*, 1948, 2138). This preparation, however, did not proceed smoothly, and (I) was not obtained completely pure. Alternative syntheses have, therefore, been carried out by applying the Elbs persulphate oxidation to 5-hydroxy-4′-methoxyflavone (II) and to 8-*hydroxy-4′-methoxyflavone* (III). Seshadri and his co-workers have used this reaction for the synthesis of a wide variety of hydroxylated 5 : 8-dihydroxyflavones and related compounds, by introducing a new hydroxyl group into position 8 of a 5-hydroxy-derivative (for bibliography and general discussion see Baker and Brown, *J.*, 1948, 2303), but the introduction of a hydroxyl group into position 5 of an 8-hydroxy-compound has not hitherto been effected.



Difficulty was experienced in preparing 5-hydroxy-4′-methoxyflavone (II) by fusion of 2 : 6-dihydroxyacetophenone with anisic anhydride and sodium anisate, followed by hydrolysis of the product, according to Syed and Wheeler (*J.*, 1936, 1714). These authors do not record the yield, but we find that it does not exceed 12%. When the fusion product was worked up without hydrolysis, it was found to contain 5-*hydroxy-4′-methoxy-3-anisoylflavone* in at least 85% yield. This compound is difficult to characterise owing to complicated polymorphic phenomena, but it readily yields a homogeneous *acetyl* derivative. Attempts were made to remove the 3-anisoyl group by alkaline hydrolysis under a variety of conditions using, *e.g.*, sodium carbonate or sodium hydroxide in aqueous alcohol, but the flavone was found to be very largely destroyed, and only very small quantities of the desired 5-hydroxy-4′-methoxyflavone (II) could be isolated. It is clear that rupture of the heterocyclic ring occurs more readily than hydrolysis of the 3-anisoyl group; this is contrary to general experience and to the very closely related case of 5-hydroxy-3-benzoylflavone which readily loses the benzoyl group alone by alkaline hydrolysis (Rajagopalan, Rao, and Seshadri, *Proc. Indian Acad. Sci.*, 1947, **25**, 436).

Oxidation of 5-hydroxy-4′-methoxyflavone with potassium persulphate in alkaline, aqueous pyridine solution gave 5 : 8-dihydroxy-4′-methoxyflavone. The identity of this product with that previously prepared was confirmed by the mixed melting points of the diacetates and the dimethyl ethers. A by-product of the oxidation of (II) was isolated as its acetyl derivative; this is undoubtedly 5 : 6 : 8-*triacetoxy-4′-methoxyflavone* (IV) arising from the introduction of two hydroxyl groups; this appears to be the first recorded case of the introduction of two hydroxyl groups during the Elbs persulphate oxidation. The new groups are assumed to be in positions 6 and 8, *ortho* and *para* to the original hydroxyl group in position 5, because the intermediate phenyl potassium sulphate grouping in position 8 or 6 is likely to be weakly *ortho–para* directing compared with the free hydroxyl group in position 5.

5-Hydroxy-4′-methoxy-3-anisoylflavone was similarly oxidised to 5 : 8-*dihydroxy-4′-methoxy-3-anisoylflavone*, and in this case also a trihydroxy-compound was isolated as its acetyl derivative, which, for the above reasons, is assumed to be 5 : 6 : 8-*triacetoxy-4′-methoxy-3-anisoylflavone*.

8-Hydroxy-4′-methoxyflavone (III) was prepared by fusing 2 : 3-dihydroxyacetophenone with anisic anhydride and sodium anisate, and subsequently hydrolysing the product. When

the fusion mixture was worked up without hydrolysis, two products were isolated, 8-*anisoyloxy*-4′-*methoxy*-3-*anisoylflavone* which could be hydrolysed to 8-hydroxy-4′-methoxyflavone, and a smaller amount of 8-*anisoyloxy*-4′-*methoxyflavone*. The formation of 3-acylflavones is a general feature of the Allan–Robinson method and was investigated by Baker (*J.*, 1933, 1383). Oxidation of 8-hydroxy-4′-methoxyflavone (III) with potassium persulphate under the usual conditions gave 5 : 8-dihydroxy-4′-methoxyflavone (I), but in much smaller yield than by the oxidation of the isomeric 5-hydroxy-4′-methoxyflavone (II).

It may be noted that the melting points of pure 5 : 8-dihydroxy-4′-methoxyflavone (233·5—234°) and its diacetyl derivative (231·5°) are very close to those recorded by Furukawa (*loc. cit.*) for ginkgetin (238—240°) and diacetylginkgetin (226—228°). The dimethyl ethers of 5 : 8-dihydroxy-4′-methoxyflavone and of ginkgetin are, however, widely different, the former having m. p. 162·5° and the latter m. p. 225—227°.

Experimental.

5-*Hydroxy*-4′-*methoxy*-3-*anisoylflavone*.—2 : 6-Dihydroxyacetophenone (8 g.), anisic anhydride (40 g.), and sodium anisate (12 g.) were fused and stirred at 190—205° for 6 hours. The product was boiled with water (75 c.c.), ethyl acetate added under reflux until complete solution occurred, and the mixture cooled and then shaken with sodium carbonate (20 g.) and water (200 c.c.) for 18 hours. Filtration yielded brownish-yellow crystals (11·5 g.), m. p. 156—157·5°, and from the ethyl acetate layer were obtained yellow prisms (4·2 g.), m. p. 121—133°. These products were combined and recrystallised twice from ethyl alcohol (1 l.), giving yellow prisms (7·4 g.), melting at 151—152° to a cloudy fluid which cleared at 158°. Further crystallisation gave a mixture of bright yellow prisms, m. p. 160—161°, and pale yellow prisms of indeterminate melting point. The more compact, bright yellow prisms were separated by taking advantage of their rapid settling from alcoholic suspension, and on recrystallisation from alcohol gave the pale yellow prisms, m. p. *ca.* 152—154° [Found : C, 71·3; H, 4·4; OMe, 15·5. $C_{22}H_{18}O_4(OMe)_2$ requires C, 71·6; H, 4·5; OMe, 15·4%]. This 5-*hydroxy*-4′-*methoxy*-3-*anisoylflavone* is clearly polymorphic and exhibits a variety of melting points depending on the solvent used for its crystallisation, and on the temperature of the bath on introduction of the specimen. Apart from the form, m. p. 160—161° (usually obtained from ethyl acetate), two other melting points are observed in the neighbourhood of 142° and 174°. With an original bath-temperature of 100°, the substance (m. p. *ca.* 152—154°) undergoes partial melting at *ca.* 152° but does not clear till 174°; with an original temperature of 135° it melts at *ca.* 173°; at 142° it melts rapidly and completely. The compound gives a transient green coloration with alcoholic ferric chloride, which rapidly changes to a brownish-purple.

5-*Acetoxy*-4′-*methoxy*-3-*anisoylflavone*.—The mixture of dimorphic forms of 5-hydroxy-4′-methoxy-3-anisoylflavone (1·5 g.) was heated under reflux for 5 hours with acetic anhydride (15 c.c.), excess of reagent was removed by distillation under diminished pressure, and the residue treated with water, yielding a homogeneous solid (1·45 g.), m. p. 218—219° without crystallisation. By dissolution in ethyl acetate (175 c.c.) and concentration (to 60 c.c.), colourless diamond-shaped prisms (1·2 g.), m. p. 220°, were obtained (Found : C, 70·5; H, 4·5. $C_{26}H_{20}O_7$ requires C, 70·3; H, 4·5%). Hydrolysis of this 5-*acetoxy*-4′-*methoxy*-3-*anisoylflavone* by boiling with a mixture of acetic and hydrochloric acid for half an hour readily regenerated the parent hydroxy-compound.

5 : 8-*Dihydroxy*-4′-*methoxyflavone* (I).—5-Hydroxy-4′-methoxyflavone (1 g., 1 mol.; m. p. 152—153°) was dissolved in a mixture of pyridine (20 c.c.) and water (30 c.c.) containing potassium hydroxide (1·05 g., 5 mols.), and to the stirred solution was added during 2 hours a solution of potassium persulphate (1·5 g., 1·5 mols.) in water (40 c.c.). After 24 hours the solution was acidified to Congo-red [unchanged 5-hydroxy-4′-methoxyflavone (0·45 g.) was thereby precipitated], and the filtrate was extracted twice with ether and then heated for half an hour on the water-bath after the addition of sodium sulphite (3 g.) and concentrated hydrochloric acid (35 c.c.). After the mixture had cooled, the yellow, crystalline solid (0·25 g.) was collected and crystallised four times from ethyl alcohol * (once with charcoal), giving fine, yellow prisms (0·1 g.), m. p. 233·5—234° (Found : C, 67·6; H, 4·5; OMe, 11·1. Calc. for $C_{15}H_9O_4{\cdot}OMe$: C, 67·6; H, 4·2; OMe, 10·9%). The diacetyl derivative formed colourless, microcrystalline prisms, m. p. 231·5° (from ethyl alcohol), which showed no depression of the melting point when mixed with 5 : 8-diacetoxy-4′-methoxyflavone, m. p. 230°, prepared as previously described by Baker and Flemons (*J.*, 1948, 2138). Further identification was provided by vigorous methylation, with methyl sulphate and potassium hydroxide in aqueous acetone, to 5 : 8 : 4′-trimethoxyflavone, m. p. and mixed m. p. with an authentic specimen (Baker and Simmonds, *J.*, 1940, 1373; Baker and Flemons, *loc. cit.*) 162·5°.

5 : 6 : 8-*Triacetoxy*-4′-*methoxyflavone* (IV).—The alcoholic mother-liquors from the crystallisation of the 5 : 8-dihydroxy-4′-methoxyflavone above * were evaporated and heated under reflux with acetic anhydride and a drop of pyridine for 1 hour. The solid precipitated on the addition of water was crystallised four times from alcohol (once with charcoal), giving colourless, microcrystalline prisms (17 mg.), m. p. 248—248·5° after sintering at 247° [Found : C, 61·9; H, 4·4; Ac, 34·7.† $C_{16}H_9O_3(OAc)_3$ requires C, 61·9; H, 4·2; Ac, 30·3%]. When mixed with 5 : 8-diacetoxy-4′-methoxyflavone, m. p. 230°, the melting point was *ca.* 218°. A larger quantity of this 5 : 6 : 8-*triacetoxy*-4′-*methoxyflavone* was isolated from a number of oxidation experiments, and, after twelve recrystallisations from alcohol, the fine needles had a constant m. p. of 253·5—254°. Hydrolysis to the trihydroxy-compound was attempted using acetic acid–hydrochloric acid, but the product darkened and was not isolated in the pure state; attempts to convert it into a flavoquinone by oxidation with *p*-benzoquinone were unsuccessful. The high value for the above acetyl determination † is typical of other cases (not quoted) in this series, and is undoubtedly due to breakdown of the heterocyclic nucleus under the conditions of the determination.

5 : 8-*Dihydroxy*-4′-*methoxy*-3-*anisoylflavone*.—A solution of 5-hydroxy-4′-methoxy-3-anisoylflavone

(2·5 g., 1 mol.) in water (32 c.c.) and pyridine (15 c.c.) containing potassium hydroxide (1·74 g., 5 mols.) was oxidised by the addition of potassium persulphate (1·68 g., 1 mol.) in water (60 c.c.), and the product worked up as in the case of the oxidation of 5-hydroxy-4′-methoxyflavone (above). The crude material (0·1 g.) was crystallised four times from ethyl alcohol (charcoal), giving yellow prisms (30 mg.), m. p. 233·5—234° (Found: C, 66·9; H, 4·8. $C_{24}H_{16}O_7,H_2O$ requires C, 66·1; H, 4·6%). This 5 : 8-*dihydroxy-4′-methoxy-3-anisoylflavone* has the same melting point as 5 : 8-dihydroxy-4′-methoxyflavone, but a mixed melting-point determination showed a depression of 29°.

5 : 6 : 8-*Triacetoxy-4′-methoxy-3-anisoylflavone*.—The product contained in the alcoholic mother-liquors was acetylated with acetic anhydride and pyridine and, after four crystallisations from ethyl alcohol, the *triacetyl* derivative was obtained as colourless, short prisms (15 mg.), m. p. 214—215° (Found: C, 64·6; H, 4·5. $C_{30}H_{24}O_{11}$ requires C, 64·3; H, 4·3%).

2 : 6-*Dianisoyloxyacetophenone*.—This compound was previously isolated when attempting to prepare the monoanisoyl derivative of 2 : 6-dihydroxyacetophenone by interaction with 1 mole of anisoyl chloride in pyridine (Baker and Flemons, *loc. cit.*). By using 2·5 moles of the acid chloride, washing the crude product with aqueous sodium carbonate, and crystallising from alcohol, a 90% yield of the pure compound, m. p. 141°, was obtained. Molecular rearrangement to give 2-hydroxy-6-anisoyloxy-4′-methoxydibenzoylmethane could not be successfully carried out by heating with sodamide in toluene, or with potassium carbonate in toluene or pyridine.

8-*Hydroxy-4′-methoxyflavone* (III).—(*a*) 2 : 3-Dihydroxyacetophenone (4 g.; Baker and Smith, *J.*, 1936, 347), anisic anhydride (28 g.), and sodium anisate (4 g.) were stirred at 180—190° for 7 hours. The product was dissolved in alcohol (600 c.c.) and hydrolysed by heating under reflux for 10 minutes with a solution of potassium hydroxide (15 g.) in water (20 c.c.); the solution was then diluted with water (300 c.c.) and, after removal of the alcohol under diminished pressure, saturated with carbon dioxide. The solid thus obtained was dissolved in a solution of potassium hydroxide (2 g.) in water (80 c.c.), the solution shaken with ether, and the phenolic product reprecipitated by carbon dioxide. The 8-*hydroxy-4′-methoxyflavone* (III) was crystallised (charcoal), first from ethyl acetate and then from alcohol, giving fine, colourless needles (1·3 g.), m. p. 235° (Found: C, 71·2; H, 4·4; OMe, 11·4. $C_{15}H_9O_2{\cdot}OMe$ requires C, 71·6; H, 4·5; OMe, 11·6%). The *acetyl* derivative formed colourless needles, m. p. 166—167° (Found: C, 69·3; H, 4·2; OMe, 10·3. $C_{17}H_{11}O_4{\cdot}OMe$ requires C, 69·7; H, 4·5; OMe, 10·0%), from alcohol.

(*b*) 8-Anisoyloxy-4′-methoxy-3-anisoylflavone (0·9 g.; below) was dissolved in alcohol (75 c.c.) and boiled for 10 minutes with a solution of potassium hydroxide (0·2 g.) in water (2 c.c.). The solution was diluted with water (75 c.c.), freed from alcohol by distillation under diminished pressure, and saturated with carbon dioxide. The precipitated solid was collected and crystallised from ethyl alcohol, giving colourless needles (0·08 g.), m. p. 234·5°, undepressed by admixture with the material prepared according to method (*a*).

8-*Anisoyloxy-4′-methoxy-3-anisoylflavone and* 8-*Anisoyloxy-4′-methoxyflavone*.—2 : 3-Dihydroxyacetophenone (2 g.), anisic anhydride (14 g.), and sodium anisate (2 g.) were stirred at 180—190° for 7 hours. A solution of the product in hot ethyl acetate (100 c.c.) and water (20 c.c.) was cooled and shaken with a solution of sodium carbonate (10 g.) in water (100 c.c.) for 18 hours. The pale yellow solid which separated was collected (3·1 g.) and digested with boiling alcohol. From the alcoholic solution was obtained 8-*anisoyloxy-4′-methoxy-3-anisoylflavone*, which on further crystallisation separated as pale yellow plates (1·8 g.), m. p. 164—166° [Found: C, 71·5; H, 4·8; OMe, 18·2. $C_{29}H_{18}O_5(OMe)_3$ requires C, 71·6; H, 4·5; OMe, 17·4%]. The alcohol-insoluble 8-*anisoyloxy-4′-methoxyflavone* (0·6 g.) separated from ethyl acetate as colourless plates, m. p. 204—205° [Found: C, 71·6; H, 4·6; OMe, 15·9. $C_{23}H_{12}O_4(OMe)_2$ requires C, 71·6; H, 4·5; OMe, 15·4%].

5 : 8-*Dihydroxy-4′-methoxyflavone* (I) *from* 8-*Hydroxy-4′-methoxyflavone* (III).—To 8-hydroxy-4′-methoxyflavone (0·5 g.) dissolved in a solution of potassium hydroxide (0·6 g.) in water (25 c.c.) was added, with stirring, during ¼ hour, potassium persulphate (0·75 g.) in water (25 c.c.). After 24 hours at room temperature, the dark solution was rendered faintly acid to Congo-red, filtered from a brown solid, and extracted with ether. The aqueous solution was then heated on a water-bath (½ hour) with sodium sulphite (1·5 g.) and concentrated hydrochloric acid (20 c.c.) and then cooled, and the solid collected, washed, and crystallised twice from alcohol (charcoal). The 5 : 8-dihydroxy-4′-methoxyflavone (I) separated as bright yellow plates (14 mg.), m. p. 233·5—234° (Found: C, 67·0; H, 4·3. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·2%), undepressed when mixed with the specimen of the same m. p., prepared by the oxidation of 5-hydroxy-4′-methoxyflavone (above). The diacetyl derivative formed needles from alcohol, m. p. and mixed m. p. 232°.

2-*Hydroxy-3-anisoyloxyacetophenone*.—To a solution of 2 : 3-dihydroxyacetophenone (0·5 g.) in dry pyridine (10 c.c.) cooled in ice-salt, was added anisoyl chloride (1·68 g.), and the mixture was slowly allowed to reach room temperature. Next day it was added to excess of dilute hydrochloric acid, and the sticky solid collected and crystallised from methyl alcohol, giving faintly yellow aggregates of cubes showing parallel growth (0·26 g.), m. p. 152—153° (Found: C, 66·6; H, 4·9; OMe, 10·4. $C_{15}H_{11}O_4{\cdot}OMe$ requires C, 67·1; H, 4·9; OMe, 10·8%). It gives a purple colour with alcoholic ferric chloride.

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THE UNIVERSITY, BRISTOL. [*Received, January 21st*, 1949.]

334. *The Chemistry of Fungi. Part VII. Syntheses of Citrinin and Dihydrocitrinin.*

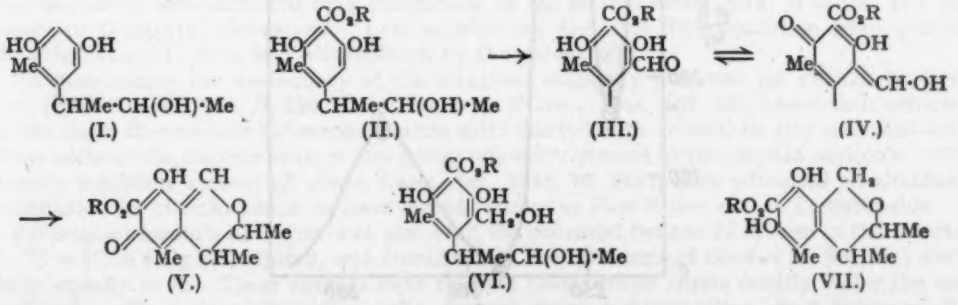
By N. J. Cartwright, Alexander Robertson, and W. B. Whalley.

The carboxylation of the lævorotatory phenol (A) (I) furnished the acid (II; R = H) and on being subjected to the Gattermann reaction this acid and its methyl ester gave rise to products which are undoubtedly (III; R = H) and (III; R = Me) and which, on cyclisation with sulphuric acid, are converted into citrinin and its methyl ester respectively, identical in every way with natural specimens. By the same procedure optically inactive citrinin has been synthesised from phenol (B).

The interaction of the ester (II; R = Me) with formaldehyde in the presence of aqueous sodium hydroxide was accompanied by hydrolysis of the carbomethoxy-group, giving dihydrocitrinin (VII; R = H) in good yield, presumably by way of the intermediate type (VI).

We have been unable to obtain evidence that 2 : 4-dichlorobenzenediazonium sulphate couples with citrinin to form an azo-dye without the extrusion of any of the groups, and consequently we consider that the claim of Gore *et al.* (*Nature*, 1946, **157**, 333) cannot be maintained.

In deducing a revised structure (V; R = H) for citrinin (Part V, this vol., p. 867) we directed attention to the fact that the new formula was an anhydro-form of (III; R = H) and suggested that a compound having the latter structure might possibly be an intermediate in the phytochemical production of citrinin. Although the latter process is still obscure, we have now succeeded in effecting a synthesis of citrinin and its methyl ester by way of intermediates type (III), thus obtaining conclusive evidence in support of the new formula. Starting with the *lævo*-form of 3-(4 : 6-dihydroxy-*o*-tolyl)butan-2-ol (I), which is readily accessible from citrinin and which for the sake of brevity and convenience we shall continue to refer to as phenol (A) (compare Part IV, this vol., p. 859), we prepared the lævo-*acid* (II; R = H) by the carboxylation process employed for the synthesis of *p*-orsellinic acid (Robertson and Robinson, *J.*, 1927, 2196). The orientation of this acid (II; R = H) follows from its method of preparation, from its intense blue ferric reaction characteristic of γ-resorcylic acids, and from the ultimate conversion of the compound into citrinin. On being subjected to the Gattermann reaction, the acid (II; R = H) gave rise to a bright yellow oily product which was not further purified but which is essentially the aldehyde (III; R = H). The failure of this substance to react with carbonyl reagents is not unexpected and is considered to be due either to the steric effects of the groups adjacent to the formyl residue or to the existence of the compound in the hydroxymethylene form, type (IV), as in the case of *m*-xylorcylaldehyde (compare Robertson and Robinson, *loc. cit.*). On being dehydrated with concentrated sulphuric acid at room temperature the crude formyl derivative was smoothly converted into citrinin in good yield, identical in every way with the natural substance, and having almost the same specific lævorotation.



By the same procedure the *methyl* ester (II; R = Me) of the *lævo*-acid (II; R = H) was converted into methyl citrinin, identical with the natural derivative and showing the same characteristic tendency to retain solvent of crystallisation to which attention was directed in Part V (*loc. cit.*) when the compound was first described. We have also been able to record a new and higher value for the specific rotation (dextro-) of the ester. The lower rotation previously observed for the natural derivative would appear to have been due to the partial decomposition of the compound during the preparation of the required standard chloroform solution by warming, whereas in the present instance the solvent employed was acetone at room temperature, in which the ester is stable. We have found that methyl citrinin decom-

poses slowly in warm alcohol and more rapidly in cold impure chloroform containing traces of hydrogen chloride; when observed in a polarimeter the dextrorotatory solution of the ester in the latter solvent is seen to undergo a change in rotation as a result of hydrolysis and formation of the lævorotatory parent acid. On being boiled, a colourless solution of methyl citrinin in 95% alcohol, prepared at room temperature, becomes yellow owing to the decomposition of

FIG. 1.

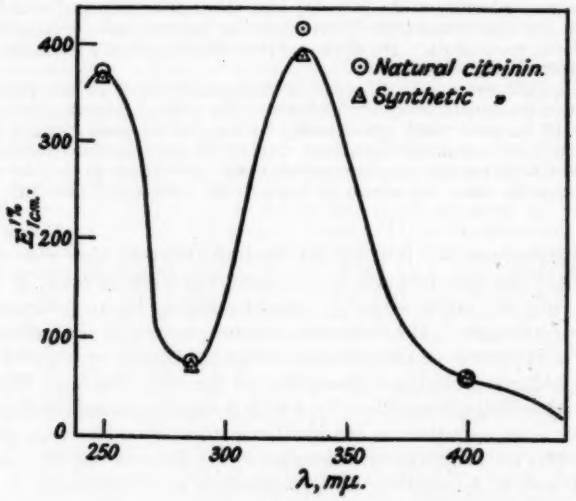
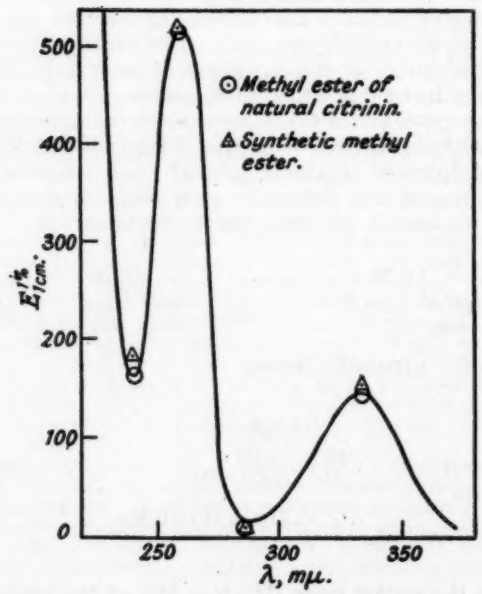


FIG. 2.



the ester; the considerable losses incurred in the purification of this ester from aqueous solvents arise mainly in this way.

From phenol (B), which is an optically inactive isomeride of phenol (A), an optically inactive citrinin has been prepared by the route employed for citrinin and its methyl ester and differs only from the natural compound in having a zero rotation. In this connection it may be noted that the synthesis of the *p*-nitrobenzoate of the dimethyl ether of phenol (B) has already been described together with the resolution of the acid phthalate of this ether to give the corresponding acyl derivative of the dimethyl ether of phenol (A) (Part IV, *loc. cit.*) which can be hydrolysed

without undergoing racemisation. We have now found that demethylation of the dimethyl ether of phenol (A) is accompanied by racemisation, yielding phenol (B), and consequently the main essential stage required to complete the synthesis of optically inactive citrinin has been completed. The remaining stage required to complete the synthesis of natural citrinin is either the preparation of phenol (A) by the resolution of phenol (B) or, alternatively, the resolution of synthetical inactive citrinin.

Further confirmation of the identity of natural and synthetical citrinin and their respective methyl esters was afforded by a comparison of the ultra-violet absorption spectra in alcoholic solution. The general shapes of the absorption curves for citrinin and the methyl ester are recorded in Figs. 1 and 2, respectively. The curves obtained for the natural and the synthetical substances were almost coincident, within the limits of experimental error, and to facilitate clarity of presentation only the curves for the natural compounds have been drawn. With the aid of a Beckman quartz spectrophotometer particular attention was paid to the regions near the maxima, minima, and points of inflexion.

The main data are summarised in the table, where a higher value of the extinction coefficient at 330 mμ is recorded for a rigorously purified specimen of natural citrinin (compare Part V, *loc. cit.*).

Substance.	λ, max., mμ.	λ, min., mμ.	λ, infl., mμ.	$E^{1\%}_{1\ cm.}$.
Natural citrinin	250			370
	331			418
			400	63·2
		286		77
Synthetic citrinin	250			367
	331·5			390
			400	62·1
		286		72·9
Natural methyl ester	260			520
	334			151·6
		241		187·6
		285		11·3
Synthetic methyl ester	259·5			515
	336			141·2
		241		160
		285		10

The structure (VII; R = H), which was proposed in Part V (*loc. cit.*) for dihydrocitrinin and was based on the results of degradation experiments, has now been confirmed by a synthesis of the compound. The condensation of the optically active ester (II; R = Me) with formaldehyde in aqueous sodium hydroxide is accompanied by the hydrolysis of the carbomethoxy-group and simultaneous cyclisation of the intermediate alcohol type (VI; R = H or Me), giving a good yield of dihydrocitrinin, identical with the natural substance. The identity of the two compounds was confirmed by a comparison of the methyl esters (VII; R = Me) and their respective *O*-diacetyl derivatives. Less satisfactory yields of dihydrocitrinin were obtained when the ester (II; R = Me) was replaced by the parent acid.

In maintaining the inadequacy of the structure originally proposed for citrinin by Coyne *et al.* (*Phil. Trans.*, 1931, *B*, **220**, 301), Gore *et al.* (*Nature*, 1946, **157**, 333) based their criticisms on the claim that certain benzenediazonium salts reacted with citrinin to give monoazo-derivatives without the displacement of any group originally present in the citrinin molecule. More recently the latter authors (*J. Amer. Chem. Soc.*, 1948, **70**, 2287) have advanced an alternative constitution for citrinin which we have already shown in Part V (*loc. cit.*) to be untenable. As in the original formula of Coyne *et al.* (*loc. cit.*), the potential benzenoid system in the structure (V; R = H) is fully substituted, and consequently the criticisms of Gore *et al.* (*loc. cit.*) should apply equally to it. These authors have claimed that citrinin reacts readily under the usual conditions with benzenediazonium salts derived from *o*-chloroaniline, 2 : 5-dichloroaniline, and sulphanilamide, yielding monoazo-dyes, but they describe only the 2 : 5-dichloro-azo-derivative. They further state that with diazotised aniline the reaction is complex and that the azo-derivative of citrinin, m. p. 182°, was accompanied by the bisazo-derivative of phenol (A), m. p. 205° (decomp.). It is now clear that the claims of these workers cannot be sustained unless it is assumed that the methine group of the residue ·CH·O· of (V; R = H) is concerned in the coupling reaction, a possibility which we are unable to support. Further, with the somewhat scanty experimental details described by Gore *et al.* as a guide, we have investigated the action of 2 : 5-dichlorobenzenediazonium sulphate on citrinin under a variety of conditions and have completely failed to find any evidence of the formation of a compound corresponding

to the 2 : 5-dichlorobenzeneazo-derivative of citrinin, m. p. 201°. Instead, we obtained the *bisazo*-derivative of phenol (A), m. p. 280° (decomp.) [identical with a specimen prepared from authentic phenol (A)], along with a product, m. p. 245° (decomp.), the composition of which is not in agreement with that required by the corresponding monoazo-derivative of citrinin; it may be noted that the bis-2 : 5-dichlorobenzeneazo-derivative of phenol (B) has m. p. 289° (decomp.). When benzenediazonium sulphate was employed we were unable to isolate a homogeneous substance from the complex reaction mixture.

Experimental.

Demethylation of lævo-3-(4 : 6-*Dimethoxy-o-tolyl*)*butan-2-ol* [*Dimethyl Ether of Phenol* (*A*)].—This ether (2 g.) was gently boiled with a mixture of hydriodic acid (5 ml.) and acetic anhydride (10 ml.) containing red phosphorus (1 g.) for 5 minutes, and the cooled reaction mixture was diluted with water (100 ml.), neutralised with sodium hydrogen carbonate, and extracted with ether (100 ml. × 5). The combined ethereal solutions were then extracted with 2N-aqueous sodium hydroxide (50 ml. × 3), the alkaline extracts were acidified, and optically inactive 3-(4 : 6-dihydroxy-*o*-tolyl)butan-2-ol, phenol (B), was isolated with ether (wash with sodium hydrogen sulphite). Crystallised from chloroform, phenol (B) formed colourless prisms (200 mg.), m. p. 169—170°, undepressed on admixture with a specimen prepared from citrinin. Evaporation of the residual ethereal solutions which had been washed with alkali gave unchanged dimethyl ether (1·4 g.). When the time of heating of the demethylation mixture was increased from 5 to 15 minutes the product consisted of phenolic material from which only traces of phenol (B) could be separated. Demethylation by means of pyridine hydrochloride at 150—170° for 3 hours likewise gave only traces of the required phenol.

lævo-3-(4 : 6-*Dihydroxy-5-carboxy-o-tolyl*)*butan-2-ol* (II; R = H).—An intimate mixture of phenol (A) (3 g.), potassium hydrogen carbonate (20 g.), and glycerol (30 ml.) was kept at 150—155° for 7 hours in an atmosphere of carbon dioxide, cooled, treated with water (150 ml.), saturated with ammonium sulphate, and extracted with ether (100 ml. × 6) to remove a small quantity of unchanged phenol. The aqueous liquors were then acidified with hydrochloric acid and the *acid* (2·25 g.) isolated by extraction with ether (100 ml. × 6) and crystallised from benzene, forming colourless squat prisms, m. p. 185° (decomp.) (Found, in a specimen dried in a vacuum at 60° for 2 hours: C, 60·1; H, 6·3. $C_{12}H_{16}O_5$ requires C, 60·0; H, 6·7%), $[\alpha]_D^{18°}$ −38·2° (*c*, 2·83 in alcohol), readily soluble in methanol or alcohol and sparingly soluble in benzene or light petroleum and giving an intense blue ferric reaction in alcohol.

A solution of this acid (2 g.) in a mixture of ether (75 ml.) and methanol (25 ml.) was treated with slightly more than one molecular proportion of ethereal diazomethane and the crystalline product was dissolved in 2N-aqueous sodium hydroxide (20 ml.) at 0° to remove diazomethane retained by the solid. Acidification of this solution with hydrochloric acid followed by extraction with ether gave the *methyl* ester, which was purified by distillation in a high vacuum and obtained as a colourless oil (1·8 g.), b. p. 164—166°/0·1 mm., $[\alpha]_D^{18°}$ −38·5° (*c*, 3·996 in methanol), having a greenish-blue ferric reaction in alcohol (Found: C, 61·1; H, 7·5. $C_{13}H_{18}O_5$ requires C, 61·4; H, 7·1%).

Carboxylation of phenol (B) by the procedure employed for the phenol (A) gave rise to the optically inactive *acid* (II; R = H), forming colourless stout prisms, m. p. 175—176° (decomp.), exhibiting the same ferric reaction (Found, in a specimen dried in a vacuum at 100°: C, 60·4; H, 6·3%).

Optically Inactive Citrinin (V; R = H).—A solution of the optically inactive acid (II; R = H) (1·1 g.) and hydrogen cyanide (5 ml.) in ether (125 ml.), containing zinc chloride (1 g.), was saturated at 0° with hydrogen chloride, and 48 hours later the crystalline product was collected, well washed with ether, and dissolved in cold water (50 ml.). This solution, which had been almost neutralised with ammonia, was heated at 80—90° for 10 minutes, cooled, and extracted with ether (50 ml. × 5). The acidic product was separated from the combined ethereal extracts by means of aqueous sodium hydrogen carbonate (50 ml. × 3), and after acidification of the latter solution this compound was isolated with ether and obtained as a pale yellow oil which did not solidify and had a blue-green ferric reaction in alcohol. A solution of this material in cold concentrated sulphuric acid (10 ml.) was kept at room temperature for 10 minutes and then poured on ice (50 g.). The resulting solid was collected, washed, dried, and crystallised from methanol, giving *inactive citrinin* in bright yellow prisms (0·4 g.), m. p. 175° (decomp.), which exhibited the iodine-brown ferric reaction characteristic of natural citrinin (Found: C, 62·2; H, 5·4. $C_{13}H_{14}O_5$ requires C, 62·4; H, 5·6%).

Citrinin (V; R = H).—The condensation of the *lævo*-acid (II; R = H) (1·2 g.) with hydrogen cyanide (2·5 ml.) by means of zinc chloride (2 g.) and excess of hydrogen chloride in ether (50 ml.), according to the procedure employed for the inactive isomeride, gave an oily condensation product, which on treatment with sulphuric acid (10 ml.) at room temperature for 15 minutes furnished citrinin (0·4 g.). Crystallised from alcohol, the synthetical compound formed characteristic yellow needles, m. p. and mixed m. p. 175° (decomp.), $[\alpha]_D^{18°}$ −37·4° (*c*, 1·15 in alcohol) (Found: C, 62·2; H, 5·6%). The m. p. of citrinin, which is a decomposition point, varies slightly with the rate of heating of the specimen. Rapid heating gives m. p. 178—179° (decomp.) and slower heating gives m. p. 175° (decomp.).

When the methyl ester (2·2 g.) of the (−)-acid (II; R = Me) was employed in place of the parent acid (II; R = H) in the Gattermann reaction the product was a yellow oil which on treatment with sulphuric acid gave rise to the methyl ester of citrinin. Recrystallised from benzene, this compound formed colourless diamond-shaped plates (1·5 g.), m. p. 139° (decomp.), $[\alpha]_D^{18°}$ +217·1° (*c*, 0·380 in acetone) (Found: C, 63·4; H, 6·8%). This product was identical in every way with a specimen of the natural ester, m. p. 139° (decomp.), $[\alpha]_D^{18°}$ +211·3° (*c*, 1·0894 in acetone), which had been recrystallised from benzene (Found: C, 63·0, 63·3, 63·1; H, 6·8, 7·0, 6·8. Calc. for $C_{14}H_{16}O_5$: C, 63·6; H, 6·1%).

Dihydrocitrinin (VII; R = H).—A solution of the methyl ester of the (−)-acid (II; R = Me) (1·5 g.) in cold 2N-aqueous sodium hydroxide (10 ml.) was treated with 40% formalin solution (10 ml.), and the orange mixture kept at room temperature for 24 hours, acidified with 2N-hydrochloric acid, and extracted

several times with ether. Evaporation of the combined washed and dried extracts left a residue which on crystallisation from benzene gave dihydrocitrinin (1 g.) contaminated with some of the acid (II; R = H). The product was extracted with chloroform in which this acid (II, R = H) is insoluble, and the residue left on evaporation of the chloroform extract was repeatedly crystallised from benzene, giving dihydrocitrinin in rosettes of colourless prisms (0·5 g.), m. p. 171° (decomp.), undepressed on admixture with a specimen of the natural derivative, $[\alpha]_D^{18°}$ −18·8° (*c*, 4·148 in chloroform) (Found: C, 62·0; H, 6·6. Calc. for $C_{13}H_{16}O_5$: C, 61·9; H, 6·4%). This compound gave the blue ferric reaction in alcohol characteristic of the natural derivative. Esterification of dihydrocitrinin with diazomethane furnished the methyl ester, forming characteristic, colourless, flat prisms, m. p. 60°, identical with a specimen from natural sources (Found: C, 62·9; H, 6·7. Calc. for $C_{14}H_{18}O_5$: C, 63·2; H, 6·8%). Acetylation of the synthetical methyl ester (100 mg.) with acetic anhydride (2 ml.) and pyridine (3 ml.) on a steam-bath for 35 minutes gave rise to the *diacetate* which separated from aqueous methanol in colourless needles (100 mg.), m. p. 114°, identical with a specimen prepared from the natural ester (Part V, *loc. cit.*) (Found: C, 61·9; H, 6·1. $C_{18}H_{22}O_7$ requires C, 61·7; H, 6·3%).

Reaction of Citrinin with 2 : 5-Dichlorobenzenediazonium Sulphate.—Prepared from dichloroaniline (1·0 g.), dissolved in concentrated sulphuric acid (15 ml.) and water (15 ml.) at −5°, a solution of the diazonium salt was added drop-wise to a well stirred solution of citrinin (2·0 g.) in 2N-aqueous sodium hydroxide (270 ml.) at 0°; 15 minutes later the reaction mixture was acidified, and the resulting precipitate washed free from acid, ground with aqueous sodium hydrogen carbonate to remove acidic material, washed, and dried. Acidification of the aqueous sodium hydrogen carbonate liquors gave only unchanged citrinin (0·3 g.). Extraction of the carbonate-insoluble product with alcohol left a fraction insoluble in the latter solvent which on crystallisation from chloroform gave the *bis*-2 : 5-*dichlorobenzeneazo*-derivative of phenol (A) in rust-coloured needles, m. p. 280° (decomp.), identical with an authentic specimen [Found: N, 10·4; Cl, 25·9. $C_{23}H_{20}O_3N_4Cl_4$ requires N, 10·3; Cl, 26·2%. Calc. for the monoazo-derivative of (A), $C_{17}H_{18}O_3N_2Cl_2$: N, 7·6; Cl, 19·3%. Calc. for the monoazo-derivative of citrinin, $C_{19}H_{16}O_5N_2Cl_2$: N, 6·6; Cl, 16·8%].

Evaporation of the alcoholic extract of the crude dye left only an intractable tar, but evaporation of the chloroform liquors from the purification of the bisazo-compound and subsequent addition of alcohol to the residue gave a *product*, m. p. 245—248° (decomp.), which on recrystallisation from chloroform–alcohol, formed glistening deep red prisms, m. p. 248° (decomp.) (Found: N, 10·2; Cl, 23·4%). In this experiment and in a number of similar experiments carried out under a variety of conditions we failed to isolate the compound, m. p. 200—201° (decomp.), described by Gore *et al.* (*loc. cit.*).

An authentic sample of the bis-2 : 5-dichlorobenzeneazo-derivative of phenol (A) was prepared by the interaction of this phenol and 2 : 5-dichlorobenzenediazonium sulphate and on purification from acetic acid or chloroform formed rust-coloured needles, m. p. 280° (decomp.) (Found: N, 10·5; Cl, 26·0%). The *bis*-2 : 5-*dichlorobenzeneazo*-derivative of phenol (B) was prepared in the same way, and on crystallisation from a comparatively large volume of acetic acid was obtained in rust-coloured needles, m. p. 289° (decomp.) (Found: N, 10·5; Cl, 26·5%).

We are greatly indebted to Professor R. A. Morton, of the Department of Biochemistry of this University, for the spectrographic data recorded in this communication.

UNIVERSITY OF LIVERPOOL. [*Received, December 8th*, 1948.]

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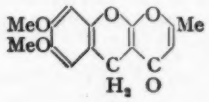
335. *The Chemistry of Fungi. Part VIII. The Oxidation of Methylene Groups in Compounds Analogous to O-Dimethylcitromycin.*

By G. W. K. CAVILL, ALEXANDER ROBERTSON, and W. B. WHALLEY.

The behaviour of diphenylmethane, fluorene, deoxybenzoin, and a number of xanthens on oxidation with ozone and with lead tetra-acetate has been compared with that of *O*-dimethylcitromycin. The rapid oxidation of the xanthens and of anthrone is closely parallel to that of *O*-dimethylcitromycin.

IN the course of experiments on the degradation of citromycetin and its derivatives (Part III, this vol., p. 848) it was discovered that *O*-dimethylcitromycin (I) was oxidised with ozone directly without the intervention of an intermediate ozonide, giving rise to *O*-dimethylcitromycinol (II) and *O*-dimethylcitromycinone (III), the latter of which is also formed when chromic oxide is used as the oxidising agent. Since this action of ozone in converting a reactive methylene group into a carbonyl group appeared to be entirely novel and because the evidence advanced for the structure of citromycetin and its derivatives depends largely on the results obtained from the hydrolytic fission of *O*-dimethylcitromycinone, it seemed desirable to examine, at least in a qualitative manner, the behaviour of compounds having structures analogous to that proposed for the citromycetin series towards ozone under conditions similar to those employed for the production of *O*-dimethylcitromycinone. Since synthetical compounds of the *O*-dimethylcitromycin type (I) are not readily available, we selected several xanthens of type (IV) which are closely related to the latter and in which the methylene group is well known

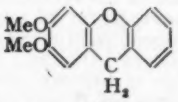
to be reactive towards oxidising agents, *e.g.* chromic oxide. At the same time we examined the behaviour of compounds, *e.g.* diphenylmethane, fluorene, and anthrone, in which the methylene group is not part of a heterocyclic oxygen system. The results have been summarised in the table below wherein the products obtained when the same compounds were oxidised with lead tetra-acetate are also given. The latter reagent has already been shown to effect substitution of a hydrogen atom in reactive methylene groups, *e.g.* in diphenylmethane (Dimroth and Schweizer, *Ber.*, 1923, **56**, 1375), in *cyclo*hexene and -indene (Criegee, *ibid.*, 1930, **481**, 263), and in *cyclo*pentene (Dane and Elder, *Annalen*, 1939, **539**, 207).



(I.)

(II.)

(III.)



(IV.)

Compound.	Oxidation products obtained with: (*a*) Ozone.	(*b*) Lead tetra-acetate.
1. Diphenylmethane	Benzophenone and glyoxal	Diphenylcarbinol acetate (*loc. cit.*)
2. Fluorene	Fluorenone	Fluorenone
3. Anthrone	Anthraquinone	—
4. Deoxybenzoin	Benzoic acid and glyoxal	Benzoin acetate
5. Xanthen	Xanthhydrol and xanthone	Xanthhydrol and xanthone
6. 1-Methyl-7-*iso*propylxanthen	The xanthhydrol	The xanthhydrol
7. 1 : 7-Dimethoxyxanthen	The xanthone	The xanthhydrol and the xanthone
8. 2 : 3-Dimethoxyxanthen	The xanthone	The xanthhydrol and the xanthone
9. *O*-Dimethylcitromycin	*O*-Dimethylcitromycin-ol and -one	*O*-Dimethylcitromycinol
10. *O*-Dimethylcitromycinol	*O*-Dimethylcitromycinone	—
11. Xanthhydrol	Xanthone	—

The oxidising action of ozone on diphenylmethane, fluorene, and deoxybenzoin is slow, requiring several hours' treatment before appreciable amounts of oxidation products are formed, and under these conditions the intermediate oxidation level (that of carbinol) has not been detected. With this group of compounds the oxidation with lead tetra-acetate requires to be carried out at an elevated temperature for comparatively long periods. On the other hand, anthrone, xanthen, and the substituted xanthens behave in a manner closely analogous to *O*-dimethylcitromycin and are rapidly oxidised with ozone or with lead tetra-acetate. With the former agent the conversion of the active methylene group into carbinol and/or carbonyl takes place in about 15—20 minutes under conditions comparable with those employed in the case of diphenylmethane, fluorene, and deoxybenzoin. We have also established independently that *O*-dimethylcitromycinol and xanthhydrol are converted into *O*-dimethylcitromycinone and xanthone, respectively, by means of ozone. The isolation of the xanthhydrols and not the xanthhydryl acetates by the oxidation of the xanthens with lead tetra-acetate is clearly due to the tendency of the acetates, which are in all probability the initial reaction products, to pass into the isomeric xanthylium salts and these are readily hydrolysed with water, an explanation which probably also applies in the case of *O*-dimethylcitromycinol. In this connection it is noteworthy that the oxidation of *O*-dimethylcitromycin with lead tetra-acetate affords an excellent method for the preparation of *O*-dimethylcitromycinol in quantity.

The results described in this communication serve to reinforce the arguments already adduced in support of the structure (I) for *O*-dimethylcitromycin (Part III, *loc. cit.*).

Experimental.

1-*Methyl*-4-iso*propylxanthone*.—2-(5′-Methyl-2′-*iso*propylphenoxy)benzoic acid, m. p. 102°, was prepared by the method of Lespagnol *et al.* (*Bull. Soc. chim.*, 1939, **6**, 1625) who gave m. p. 98°. On being heated under reflux with acetyl chloride (30 ml.) containing concentrated sulphuric acid (15 drops) for 10 minutes (method of Gottesmann, *Ber.*, 1933, **66**, 1168) (3 g.) this gave 1-methyl-4-*iso*propylxanthone (2·6 g.) which crystallised from aqueous alcohol forming colourless needles, m. p. 91° (Found: C, 81·0; H, 6·4. Calc. for $C_{17}H_{16}O_2$: C, 81·0; H, 6·4%) (compare Lespagnol *et al.*, *loc. cit.*, who give m. p. 85°). Reduction of this xanthone (1 g.), suspended in 95% alcohol, with sodium amalgam (from 0·4 g. of sodium and 2 g. of mercury) according to Hollemann's method (*Org. Synth.*, Coll. Vol. I, p. 554) in the course of about 15 minutes, followed by dilution of the reaction mixture with water and isolation of the product with ether, gave 1-methyl-4-*iso*propylxanthhydrol which formed colourless needles (0·8 g.), m. p. 94°, from aqueous methanol (Found: C, 79·9; H, 7·2. Calc. for $C_{17}H_{18}O_2$: C, 80·3; H, 7·1%) (compare Lespagnol, *et al.*, *loc. cit.*, who give m. p. 85°). This compound formed an orange solution of the xanthylium salt in 2N-hydrochloric acid. Prepared by Adriani's procedure (*Rec. Trav. chim.*, 1915, **35**, 180) the N-*phenyl*-N′-(1-*methyl*-4-iso*propylxanthyl*)*urea* separated from dioxan in slender

colourless needles, m. p. 221° (decomp.) (Found: C, 77·2; H, 6·7; N, 7·2. $C_{24}H_{24}O_2N_2$ requires C, 77·4; H, 6·5; N, 7·5%).

Reduction of 1-methyl-4-*iso*propylxanthone (2·0 g.) with sodium (8 g., added in portions) and boiling alcohol (100 ml.) according to Kostanecki's method (*Ber.*, 1908, **41**, 1325) and precipitation of the product with water gave 1-*methyl*-4-iso*propylxanthen* (1·6 g.), which formed colourless prisms, m. p. 40—41°, from methanol (Found: C, 85·1; H, 7·4. $C_{17}H_{16}O$ requires C, 85·7; H, 7·6%). A current of ozone and oxygen was passed into a solution of this compound (0·5 g.) in chloroform (40 ml.) during 20 minutes, and a solution of the residual brown oil, left on evaporation of the solvent in a vacuum, in a little methanol was treated with phenylurea in acetic acid, giving rise to colourless needles of the phenylurea derivative (0·2 g.), m. p. 220—221°, after purification, identical with an authentic specimen (Found: N, 7·9%). The oxidation product did not appear to contain appreciable amounts of 1-methyl-4-*iso*propylxanthone.

When a mixture (agitated) of 1-methyl-4-*iso*propylxanthen (0·4 g.), acetic acid (6 ml.), and lead tetra-acetate (1·6 g.) was kept at room temperature for 1 hour a test portion did not give a precipitate with phenylurea, but 4 hours later when the mixture was diluted with water (50 ml.) and extracted with ether evaporation of the washed and dried extract left an oil which, on treatment with phenylurea in acetic acid, gave the phenylurea derivative as slender needles (0·2 g.), m. p. 220—222° (decomp.), after recrystallisation from dioxan (Found: N, 7·6%). After the separation of this compound, evaporation of the filtrate gave an intractable product from which 1-methyl-4-*iso*propylxanthone could not be isolated.

1 : 7-*Dimethoxy-xanthhydrol and -xanthen.*—Prepared from 1 : 7-dimethoxyxanthone (Graebe and Ebrard, *Ber.*, 1882, **15**, 1677) (1 g.) by the Hollemann procedure (*loc. cit.*) 1 : 7-dimethoxyxanthhydrol formed colourless prisms (0·6 g.), m. p. 84°, from methanol (Found: C, 70·0; H, 5·6. $C_{15}H_{14}O_4$ requires C, 69·8; H, 5·4%) (cf. Zerner and von Loti, *Monatsh.*, 1913, **34**, 993, who gave m. p. 74—76°). This compound gave a violet solution with 2N-hydrochloric acid, and with phenylurea in acetic acid formed N-*phenyl*-N'-(1 : 7-*dimethoxyxanthyl*)*urea* which separated from dioxan in colourless slender needles, m. p. 232° (decomp.) (Found: C, 70·0; H, 5·8; N, 7·8. $C_{22}H_{20}O_4N_2$ requires C, 70·2; H, 5·4; N, 7·4%).

Reduction of 1 : 7-dimethoxyxanthone (1 g.) by the sodium and alcohol method yielded 1 : 7-*dimethoxyxanthen* (0·6 g.) which crystallised from methanol in colourless needles, m. p. 79° (Found: C, 74·2; H, 5·9. $C_{15}H_{14}O_3$ requires C, 74·4; H, 5·8%). Oxidation of this compound (0·2 g.) in chloroform (25 ml.) with ozone and oxygen during 20 minutes gave a dark solution from which the solvent was removed and the resinous product extracted with light petroleum (b. p. 40—60°). On recrystallisation from methanol the extracted material gave 1 : 7-dimethoxyxanthone (0·1 g.) in colourless needles, m. p. and mixed m. p. 147—149°. The phenylurea derivative of 1 : 7-dimethoxyxanthhydrol was formed when a mixture of the xanthen (0·2 g.), acetic acid (3 ml.), and lead tetra-acetate (0·8 g.) was kept at room temperature for 2 hours and a test sample (0·5 ml.) treated with phenylurea. On isolation the product (0·05 g.) had m. p. 228—230° (decomp.) undepressed on admixture with an authentic specimen (Found: N, 7·5%). The remainder of the reaction mixture was then kept for 24 hours and diluted with water (50 ml.), giving only 1 : 7-dimethoxyxanthone, which formed colourless needles (0·1 g.), m. p. and mixed m. p. 149°, from methanol. When the reaction was carried out at 40—50° 1 : 7-dimethoxyxanthhydrol could not be detected.

2 : 3-*Dimethoxyxanthone* (IV).—3 : 4-Dimethoxydiphenyl ether has been prepared by Asahina and Tanase (*Proc. Imp. Acad. Tokyo*, 1940, **16**, 297; *Chem. Abs.*, 1940, **34**, 7096) who prepared the xanthone from it by means of carboxyl chloride and aluminium chloride, but the details were not available to us and the following procedure was employed to synthesise this xanthone. The interaction of 4-bromoveratrole (7 g.) and sodium phenoxide (from 9·4 g. of phenol) in the presence of copper bronze (0·1 g.) according to the method of Ullmann and Stein (*Ber.*, 1906, **39**, 623) gave 3 : 4-dimethoxydiphenyl ether as a colourless oil (6·8 g.), b. p. 195—205°/20 mm., which solidified on being kept, and then separated from methanol in diamond-shaped plates, m. p. 50° (Found: C, 72·8; H, 6·2. Calc. for $C_{14}H_{14}O_3$: C, 73·0; H, 6·1%). A stream of hydrogen chloride was led into a stirred mixture of this ether (10 g.), benzene (40 ml.), and zinc cyanide (20 g.) at 0° for 3 hours, and aluminium chloride (16 g.) was then introduced, followed by the passage of hydrogen chloride for a further 2 hours. 3 Days later the reaction mixture was treated with ice (100 g.) and concentrated hydrochloric acid (50 ml.), and then boiled for ½ hour. On isolation with ether, the aldehyde was obtained as a viscous amber oil (6·6 g.) which gave a *semicarbazone*, forming colourless squat prisms, m. p. 210—211°, from methanol (Found: C, 61·3; H, 5·8; N, 13·3. $C_{16}H_{17}O_4N_3$ requires C, 61·0; H, 5·4; N, 13·3%). The crude aldehyde (4 g.), dissolved in acetone (100 ml.), was oxidised with powdered potassium permanganate (10 g.) added in the course of 2 hours. Next day the filtered mixture was cleared with sulphur dioxide, the acetone was distilled off, and the residue was extracted with ether. Isolated from the extracts by means of aqueous sodium hydrogen carbonate and by extraction of the precipitate of manganese dioxide with the same reagent, 2-(3' : 4'-*dimethoxyphenoxy*)*benzoic acid* (3·5 g.) was purified by repeated crystallisation from aqueous methanol with considerable loss and then from acetone–light petroleum (b. p. 40—60°), forming clusters of tiny colourless needles, m. p. 174—175° (Found: C, 65·0; H, 5·4. $C_{15}H_{14}O_5$ requires, C, 65·7; H, 5·1%). Cyclisation of the crude acid (3 g.) with boiling acetyl chloride containing sulphuric acid (15 drops) in the course of 10 minutes gave 2 : 3-dimethoxyxanthone, which formed colourless needles (2·1 g.), m. p. 164°, from methanol (Found: C, 70·4; H, 5·0. Calc. for $C_{15}H_{12}O_4$: C, 70·3; H, 4·7%). From the liquors left from the purification of the xanthone a small amount of a second acid, probably 4-(3' : 4'-dimethoxyphenoxy)benzoic acid, was obtained. This compound would arise from small amounts of the corresponding aldehyde present in the crude Gattermann product employed for the oxidation.

2 : 3-*Dimethoxy-xanthhydrol and -xanthen.*—Reduction of the foregoing xanthone (0·4 g.) with sodium amalgam furnished 2 : 3-*dimethoxyxanthhydrol* (0·3 g.), which crystallised from methanol in colourless prisms, m. p. 92° (Found: C, 69·9; H, 5·6. $C_{15}H_{14}O_4$ requires C, 69·8; H, 5·4%). This compound, which gave a deep yellow solution with 2N-hydrochloric acid and did not appear to form a phenylurea derivative readily, was characterised by being converted into 2 : 3-*dimethoxyxanthylium ferrichloride*

which formed yellow needles, m. p. 204° (decomp.), from acetic acid (Found : C, 41·3; H, 3·1; Fe, 12·7. $C_{15}H_{12}O_3,FeCl_4$ requires C, 41·0; H, 3·0; Fe, 12·7%).

Reduction of 2 : 3-dimethoxyxanthone (1 g.) with sodium and alcohol gave rise to 2 : 3-dimethoxyxanthen (0·8 g.), forming colourless prisms, m. p. 88°, from light petroleum (b. p. 40—60°) (Found : C, 74·5; H, 6·1. $C_{15}H_{14}O_3$ requires C, 74·4; H, 5·8%). On treatment with ozone and oxygen in chloroform (30 ml.) during 20 minutes this xanthen (0·2 g.) was converted into 2 : 3-dimethoxyxanthone (0·1 g.), m. p. and mixed m. p. 160°, after purification, but the presence of 2 : 3-dimethoxyxanthhydrol could not be detected in the reaction mixture.

When a solution of 2 : 3-dimethoxyxanthen (0·2 g.) in acetic acid (3 ml.) containing lead tetra-acetate (0·8 g.) had been kept for 1 hour a test portion (0·5 ml.) was diluted with water and extracted with ether. Evaporation of the combined extracts, which had been washed with aqueous sodium hydrogen carbonate, left a residue giving a deep yellow solution in 2N-hydrochloric acid characteristic of 2 : 3-dimethoxyxanthhydrol. On being kept for 24 hours the main portion of the original reaction mixture then contained only 2 : 3-dimethoxyxanthone, which formed colourless needles (0·15 g.), m. p. 160—162°, undepressed on admixture with an authentic specimen, from methanol.

The following compounds were also oxidised with ozone and with lead tetra-acetate.

(a) *Diphenylmethane.* A stream of ozone and oxygen was passed into a solution of this compound (2 g.) in chloroform (75 ml.) at 0° for 4 hours, and the product left on evaporation of the solvent in a vacuum was dissolved in methanol (30 ml.). Half of this solution, with an excess of alcoholic 2 : 4-dinitrophenylhydrazine sulphate, gave a mixed product (0·6 g.), which after having been extracted with warm acetic acid consisted mainly of glyoxal bis-2 : 4-dinitrophenylhydrazone (0·1 g.), forming scarlet needles, m. p. and mixed m. p. 320° (decomp.), from dioxan (Found : N, 25·8. Calc. for $C_{14}H_{10}O_8N_8$: N, 26·8%). On being cooled the acetic acid extracts gave benzophenone dinitrophenylhydrazone, m. p. and mixed m. p. 236°, after purification from dioxan (Found : N, 15·2. Calc. for $C_{19}H_{14}O_4N_4$: N, 15·5%). Examination of the second portion of the methanolic solution, which contained 0·5 g. of diphenylmethane, failed to reveal the presence of diphenylcarbinol.

(b) *Deoxybenzoin.* The product obtained with ozone as in the case of (a) was found to contain unchanged deoxybenzoin, benzoic acid, and glyoxal (isolated as the 2 : 4-dinitrophenylhydrazone). The addition of red lead (8·5 g.) in the course of 3 hours to a solution of deoxybenzoin (2 g.) in acetic acid (15 ml.) at 100° and subsequent dilution of the cooled reaction mixture with water gave rise to the acetate of benzoin (2 g.), forming prisms, m. p. 82°, from methanol, identified by comparison with an authentic sample.

(c) *Fluorene.* The product obtained with ozone from this compound (2 g.) consisted of unchanged material (0·8 g.) and fluorenone. The latter was isolated as the 2 : 4-dinitrophenylhydrazone (0·8 g.) forming orange-red plates, m. p. 299—300° (decomp.), from dioxan (Found : N, 15·6. Calc. for $C_{19}H_{12}O_4N_4$: N, 15·6%). The oxidation of fluorene with lead tetra-acetate in boiling acetic acid was remarkably slow, and in the course of 4 hours gave only a small amount of fluorenone, isolated as the 2 : 4-dinitrophenylhydrazone (0·1 g.), m. p. 302° (decomp.) after purification from dioxan (Found : N, 15·2%).

(d) *Xanthen.* When a stream of ozone and oxygen was led into a solution of xanthen (1 g.) in chloroform (50 ml.) for ½ hour, and the solvent evaporated in a vacuum, crystallisation of the residue from alcohol gave xanthone (0·4 g.), m. p. and mixed m. p. 174°. Treatment of the alcoholic liquor with phenylurea gave the *N*-phenyl-*N'*-xanthylurea in slender needles (0·5 g.), m. p. 230° (decomp.) (Found : N, 8·5. Calc. for $C_{20}H_{16}O_2N_2$: N, 8·9%).

A mixture of xanthen (0·45 g.), lead tetra-acetate (2·4 g.), and acetic acid was kept at room temperature for 1 hour with occasional shaking, and a test portion of the mixture then gave the *N*-phenyl-*N'*-xanthylurea (0·1 g.), m. p. 230—232° (Found : N, 8·6%). Next day the main portion of the reaction mixture was found to contain only xanthone (0·3 g.), m. p. and mixed m. p. 173°, which was precipitated with water.

(e) *Anthrone.* Treatment of this compound (2 g.) in chloroform (75 ml.) with ozone for ½ hour gave rise to anthraquinone, m. p. 265—270° after purification, which was identified by comparison with an authentic specimen. 10-Hydroxyanthrone was not formed in detectable amount.

O-Dimethylcitromycinol.—Red lead (20 g.) was added in small portions to a solution of *O*-dimethylcitromycin (5·5 g.) in acetic acid (30 ml.) at 30—40° in the course of 1 hour. ½ Hour later, the solution, which gave a negative test for lead tetra-acetate with starch–iodide paper, began to deposit the crystalline alcohol and was poured into water (100 ml.), giving a crystalline precipitate of citromycinol which after having been well washed with water and dried, had m. p. 236—238° (decomp.); yield, 4·8 g. Recrystallised from a large volume of acetone the compound formed colourless needles, m. p. 242° (decomp.) (Found : C, 62·1; H, 4·8. Calc. for $C_{15}H_{14}O_6$: C, 62·1; H, 4·8%).

When the reaction was carried out at higher temperatures, *e.g.* 70—100°, considerable decomposition occurred but *O*-dimethylcitromycinone did not appear to be formed.

It may be noted that the decomposition temperature, 242°, given here for *O*-dimethylcitromycinol differs from that recorded in Part III (*loc. cit.*). The variation is entirely due to the slower rate of the melting point determination (slow heating up to 230° and then at rate of approximately 1° per minute).

Ozone and oxygen were passed into a solution of *O*-dimethylcitromycinol (0·15 g.) in chloroform (75 ml.) for 20 minutes, and a part of the resulting *O*-dimethylcitromycinone separated. After evaporation of the solvent, the residual product was extracted with alcohol and the residue crystallised from acetic acid, giving *O*-dimethylcitromycinone (0·05 g.), pale buff needles, m. p. 315—317° (decomp.), identified by comparison with an authentic specimen.

UNIVERSITY OF LIVERPOOL. [*Received, December 28th,* 1948.]

336. *The Chemistry of the "Insoluble Red" Woods. Part III. The Structure of Santal and a Note on Orobol.*

By Alexander Robertson, C. W. Suckling, and W. B. Whalley.

An improved method has been devised for the isolation of santal, which has been found to have the empirical formula $C_{15}H_9O_5(OMe)$ originally suggested by O'Neill and Perkin and to form an *O*-triacetyl and an *O*-trimethyl derivative. By the degradation and synthesis of the latter derivative and of the corresponding triethyl ether it has been shown that santal is 5 : 3′ : 4′-trihydroxy-7-methoxy*iso*flavone (I; R = Me).

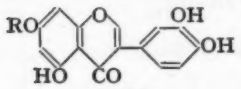
It seems reasonably certain that norsantal (I; R = H), formed by the demethylation of santal, is identical with orobol, the aglucone of oroboside (Bridel and Charaux).

The phenolic compound, santal, appears to have been first isolated in small yield from Sandelwood (syn. Red Sandelwood or Santalwood), *Pterocarpus santalinus* (Linn.), by Weidel (*Zeit. für Chem.*, 1870, **6**, 83) who assigned to it the empirical formula $C_8H_6O_3.0{\cdot}5H_2O$, but did not record a melting point. On fusing his substance with alkali, Weidel obtained piperonal and protocatechuic acid, remarking that, when kept for a long time in air, alkaline solutions of the compound became red and then green. In their examination of barwood, *Baphia nitida* (Lodd.), O'Neill and Perkin (*J.*, 1918, **113**, 125) isolated a compound, m. p. 223°, which gave the same analytical results as Weidel (*loc. cit.*) had obtained for santal, but which they, unlike Weidel who recorded a violet-black ferric reaction, found to give a wine-red ferric reaction in alcohol and to contain a methoxyl group; they proposed the empirical formula $C_{15}H_9O_5(OMe)$. It may well be that Weidel's material was largely composed of santal (compare Raudnitz and Perlmann, *Ber.*, 1935, **68**, 1862), although in the light of the results described in the present communication it is clear that the piperonal obtained from it by this author was derived from an impurity, in all probability pterocarpin (compare Part I, *J.*, 1940, 787). O'Neill and Perkin (*loc. cit.*) considered that their santal was very similar to baphic acid, one of the compounds isolated from barwood by Anderson (*Trans. Chem. Soc.*, 1876, **30**, 582), but the latter author did not define the properties of his product very clearly and the identity is not certain. Similarly, Raudnitz and Perlmann (*loc. cit.*) isolated, from sandelwood, a compound, m. p. 218°, which they considered to be santal and to have the empirical formula $C_{13}H_{10}O_5$; from their substance these authors prepared an *O*-diacetyl and an *O*-dimethyl derivative, m. p. 152° and 141°, respectively.

In the course of our examination of the colourless minor constituents of the "insoluble red" woods, sandelwood, barwood, and camwood, the formulæ of which we expect may be of some assistance in the elucidation of the structures of the more complex colouring matters, we have devised a suitable procedure for the isolation of santal, m. p. 223°, from commercial camwood which is stated to be a variety of barwood, and have also obtained the compound from sandelwood. Although at first considerable difficulty was experienced in obtaining consistent analytical results for santal and its derivatives, it finally became clear that the compound formed a *monohydrate* and that the anhydrous substance had the empirical formula $C_{15}H_9O_5(OMe)$ in agreement with that proposed by O'Neill and Perkin (*loc. cit.*). Through the kindness of Dr. Cross of the Department of Colour Chemistry, University of Leeds, we have been able to compare a specimen of O'Neill and Perkin's santal with our own material and found them to be identical.

Santal, which is devoid of a methylenedioxy- or an active keto-group, formed a *triacetate* and a *trimethyl ether*. On hydrogenation with a palladium–charcoal catalyst at 100° *O*-trimethylsantal gave rise to a compound which appears to have the formula $C_{19}H_{22}O_5$ and is devoid of a hydroxyl or carbonyl group, and which yielded a ketonic substance on oxidation with potassium permanganate. Ozonolysis of the trimethyl ether furnished a complex mixture, from which 2-hydroxy-4 : 6-dimethoxybenzoic acid was separated, together with a small amount of a crystalline compound, m. p. 77—78°, which, owing to lack of material, was not examined in detail. On being subjected to hydrolytic fission with hot aqueous or alcoholic potassium hydroxide, *O*-trimethylsantal yielded formic acid and a saturated phenolic *ketone*, $C_{14}H_8O_3(OMe)_4$ which exhibited an intense ferric reaction in alcohol and was characterised by the formation of a *methyl ether* and an *oxime*. Oxidised with potassium permanganate in acetone, this ketone gave veratric acid (characterised by the preparation of the p-*nitrobenzyl* ester) along with a ketonic *compound* which appears to have the formula $C_{14}H_8O_3(OMe)_4$. This second ketone, which was also formed in small amounts when *O*-trimethylsantal was oxidised directly with potassium permanganate in acetone, is phenolic, exhibiting a strong ferric reaction and forming (with difficulty) a *methyl ether* and a 2 : 4-*dinitrophenylhydrazone*. These properties are in keeping with the view that this substance also is an *o*-hydroxy-phenolic ketone.

From its empirical formula, its ferric reaction, and its oxidation with potassium permanganate to give veratric acid, in conjunction with the production of 2-hydroxy-4 : 6-dimethoxybenzoic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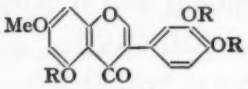
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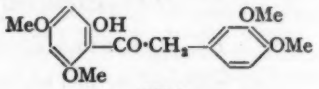
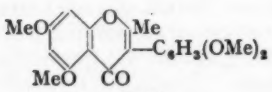

(III.)

acid by the ozonolysis of *O*-trimethylsantal, it appeared probable that the ketone $C_{14}H_8O_3(OMe)_4$ was 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (III), a conclusion which was supported by the conversion of the compound on methylation into 2 : 4 : 6 : 3′ : 4′-pentamethoxydeoxybenzoin (Freudenberg *et al.*, *Annalen*, 1925, **446**, 87). That the ketone (III) contained a hydroxyl in the *o*-position to a carbonyl group was confirmed by the fact that on vigorous acetylation with sodium acetate and acetic anhydride it underwent cyclisation giving rise to a product, $C_{20}H_{20}O_6$, which is clearly 5 : 7 : 3′ : 4′-*tetramethoxy-2-methylisoflavone* (IV). Ultimately, the structure of this ketone (III) was established synthetically by two routes. Condensation of 3 : 4-dimethoxybenzyl cyanide and phloroglucinol monomethyl ether according to the method of Hoesch gave only one product which, from the known behaviour of phloroglucinol monomethyl ether with other cyanides, is clearly 2 : 4-dihydroxy-6 : 3′ : 4′-trimethoxydeoxybenzoin (V; R and R′ = Me) and which, by partial methylation, furnished the ketone (III) identical with the product from santal. When phloroglucinol monomethyl ether is replaced by the dimethyl ether in the Hoesch reaction, the ketone (III) is obtained directly along with smaller amounts of the isomeride 4-*hydroxy*-2 : 6 : 3′ : 4′-*tetramethoxydeoxybenzoin* (VI), which, as would be expected, is readily soluble in 1% aqueous sodium hydroxide and has a negative ferric reaction in alcohol.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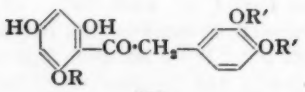
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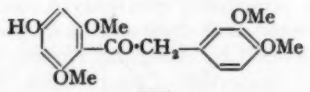
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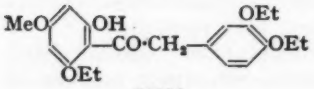
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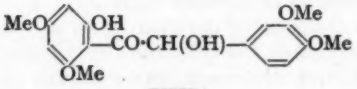
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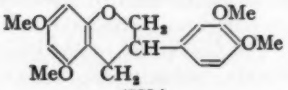
(IX.)

In view of the production of formic acid along with the ketone (III) by hydrolytic fission, it seemed reasonably certain that *O*-trimethylsantal was 5 : 7 : 3′ : 4′-tetramethoxy*iso*flavone (II; R = Me), a conclusion which was confirmed by the synthesis of the compound from the ketone (III) (natural or synthetical) and ethyl formate in the presence of sodium according to the standard procedure. Consequently, it became clear that santal was a monomethyl ether of 5 : 7 : 3′ : 4′-tetrahydroxy*iso*flavone (I; R = H). In order to determine the position of the methyl group, santal was converted into the *triethyl ether*, and on hydrolytic fission this derivative gave rise to a methoxytriethoxydeoxybenzoin, the properties of which were very similar to those of the related ketone (III). On being oxidised with potassium permanganate, the methoxytriethoxy-ketone yielded 3 : 4-diethoxybenzoic acid in addition to a ketonic compound, $C_{21}H_{26}O_6$, having an intense ferric reaction in alcohol and corresponding to the oxidation product, $C_{14}H_8O_3(OMe)_4$, obtained from the analogue (III). The production of 3 : 4-diethoxybenzoic acid clearly showed that the *O*-methyl group was in the phloroglucinol residue of the methoxytriethoxy-ketone, *i.e.*, that this ketone was 2-*hydroxy*-4-*methoxy*-6 : 3′ : 4′-*triethoxy*- (VII) or 2-*hydroxy*-6-*methoxy*-4 : 3′ : 4′-*triethoxy-deoxybenzoin*. The condensation of 3 : 4-diethoxybenzyl cyanide with phloroglucinol monoethyl ether gave rise to 2 : 4-*dihydroxy*-6 : 3′ : 4′-*triethoxydeoxybenzoin* (V; R = R′ = Et) which on partial methylation furnished the ketone (VII), identical with the compound from *O*-triethylsantal. On the other hand, the condensation of the same cyanide with phloroglucinol monomethyl ether under the same conditions, followed by monoethylation of the resulting 2 : 4-*dihydroxy*-6-*methoxy*-3′ : 4′-*diethoxydeoxybenzoin* (V; R = Me; R′ = Et), furnished 2-hydroxy-6-methoxy-4 : 3′ : 4′-triethoxydeoxybenzoin. From the synthetic ketone (VII) and methyl formate *O*-triethylsantal was synthesised by the standard method and found to be identical with the natural derivative. In consequence it became clear that triethylsantal has formula (II; R = Et) and, therefore, that santal is 5 : 3′ : 4′-trihydroxy-7-methoxy*iso*flavone (I; R = Me). In the course of the determination of the orientation of

triethylsantal the synthesis of the following *iso*flavones was undertaken simultaneously with the degradation and synthesis of *O*-triethylsantal, and their non-identity with the natural derivative serves to reinforce the arguments advanced for the structure of santal: (*a*) 5-*methoxy*-7 : 3′ : 4′-*triethoxy*i*soflavone* from 2-*hydroxy*-6-*methoxy*-4 : 3′ : 4′-*triethoxydeoxybenzoin* obtained by the partial ethylation of 2 : 4-*dihydroxy*-6-*methoxy*-3′ : 4′-*diethoxydeoxybenzoin*, (*b*) 4′-*methoxy*-5 : 7 : 3′-*triethoxy*i*soflavone* from 2-*hydroxy*-5′-*methoxy*-4 : 6 : 3′-*triethoxydeoxybenzoin*, and (*c*) 3′-methoxy-5 : 7 : 4′-*triethoxy*i*soflavone* from 2-*hydroxy*-3′-*methoxy*-4 : 6 : 4′-*triethoxydeoxybenzoin*. On the basis of the structure (II; R = Me) for *O*-trimethylsantal it seems reasonably certain that the neutral reduction product (deoxy-derivative), $C_{19}H_{22}O_5$, formed from this ether, is the *iso*flavan (IX), and hence its ketonic oxidation product is in all probability the corresponding 5 : 7 : 3′ : 4′-tetramethoxy*iso*flavanone.

It is not clear whether the diacetate and dimethyl ether of santal described by Raudnitz and Perlmann (*loc. cit.*) are impure forms of the triacetate and the trimethyl ether obtained in the present work, but it may be noted that the lower melting points observed by these authors may be due to the retention of solvents. We have observed that derivatives of santal occasionally show anomalous melting points unless precautions have been taken in those particular cases completely to remove the solvent by heating in a high vacuum. Nevertheless, for compounds having the type of structure (I) now established for santal it is well known that the hydroxyl group in the 5-position is somewhat inert towards methylating and acylating reagents under the usual conditions, and consequently it may well be that the derivatives described by Raudnitz and Perlmann are the *O*-3′ : 4′-dimethyl and the *O*-3′ : 4′-diacetyl derivatives of santal. From its method of preparation it would seem likely that their ether is the dimethyl derivative.

Whilst our investigations of the keto-phenolic oxidation product, $C_{14}H_8O_3(OMe)_4$, obtained from *O*-trimethylsantal and from 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (III) are not yet complete, it seems possible that this substance may be a benzoin derivative (VIII), but, as far as we are aware, the production of mixed benzoins in this way has not so far been described. The benzil structure is not excluded, but the failure of the compound to react with *o*-phenylenediamine makes this unlikely. Further, the alternative *p*-quinone type of structure, envisaged as being derived by oxidation of the phloroglucinol residue, is excluded because oxidation of 2 : 4 : 6 : 3′ : 4′-pentamethoxydeoxybenzoin with potassium permanganate gives small yields of a product having a negative ferric reaction and identical in every way with the *methyl ether* obtained by methylating the compound $C_{14}H_8O_3(OMe)_4$ with methyl iodide and potassium carbonate in boiling acetone. The oxidation of the analogous ketones, 2-hydroxy-4-methoxy-6 : 3′ : 4′-triethoxy-, 2-*hydroxy*-4 : 6 : 4′-*trimethoxy*- and 2-*hydroxy*-4 : 6-*dimethoxydeoxybenzoin*, gave products having properties similar to the compound $C_{14}H_8O_3(OMe)_4$. Unlike benzoin, these oxidation products reduce Fehling's solution only very slowly and, whilst they give ketonic reactions, we have not so far succeeded in preparing functional derivatives establishing the presence of a secondary alcohol group, as required by formulæ of type (VIII). Further, the compounds do not appear to be readily oxidisable with chromic oxide in acetic acid.

Orobol.—In their examination of the glucoside, oroboside, which they isolated from the vetch, *Orobus tuberosus* (L), Bridel and Charaux (*Compt. rend.*, 1930, **190**, 387) obtained from it a flavone-like aglucone, orobol, m. p. 270·5°; subsequently, Charaux and Rabate (*Bull. Soc. Chim. biol.*, 1939, **21**, 1330), prepared, from orobol, an acetyl derivative, m. p. 211° (bloc Maquenne), and obtained phloroglucinol and homoprotocatechuic acid on fusing it with potassium hydroxide, concluding that the compound was probably 5 : 7 : 3′ : 4′-tetrahydroxy*iso*flavone (I; R = H). In view of the constitution now allocated to santal it seemed likely that orobol was identical with norsantal, m. p. 270—273°, which O'Neill and Perkin (*loc. cit.*) had obtained by the demethylation of santal. We have now found that norsantal, prepared by the demethylation of santal, has m. p. 270°, and forms a tetra-acetate, m. p. 207·5°.

The following is a comparative summary of the properties of orobol as described by the French authors and of norsantal which we have observed:

	Orobol.	Norsantal.
Crystalline form	Pale-yellow, bent needles	Pale-yellow, rhombic plates
Melting point	270·5°	270°
Ferric reaction	Green, "becoming colour of diluted wine"	Green, becoming purplish brown
Sulphuric acid reaction	Yellow-green solution, becoming blood-red on addition of a few drops of concentrated nitric acid	Same as orobol
Nitric acid reaction	Brown-red solution becoming yellow	Same as orobol
Ammoniacal silver nitrate	Reduces	Reduces
Acetyl derivative	M. p. 211° (bloc Maquenne)	207·5° (tube)

Subject to a direct comparison, when a specimen of orobol becomes available, we therefore consider that the two compounds are identical.

Experimental.

Santal (I; R = Me).—Commercial " camwood " (600 g.) was heated under reflux with carbon tetrachloride (3 l.) for 6 hours, filtered, dried, and then continuously extracted in a Soxhlet apparatus with chloroform for 16 hours. After 2 further charges had been extracted, the chloroform liquor was kept for 48 hours, and the dark-red pigment (*A*) (50—80 g.) which had separated was removed by filtration. The residue left on evaporation of the chloroform filtrate in a vacuum was dissolved in ethyl acetate (1 l.) and the solution divided into 5 equal portions. Into each portion a rapid stream of dry hydrogen chloride was passed for about 10 seconds and the red slurry (*B*), which separated, was collected immediately by filtration, washed with a little ethyl acetate and then thoroughly with ether, dried, and stored for further investigation. The condition of the pigment (*B*) depended largely on the dexterity with which the operations were performed. The optimum conditions appeared to depend on the use of a dilute ethyl acetate solution of the chloroform residue, careful adjustment of the time and rate of passage of the hydrogen chloride which can only be achieved by trial, and thorough and rapid washing and drying of the solid pigment (*B*) with ether. Exposure of this solid, while moist with solvent, to the air generally resulted in the formation of dark sticky material.

The ethyl acetate filtrate and washings from the pigment (*B*) were combined, washed with a little aqueous sodium hydrogen carbonate to remove hydrogen chloride, evaporated to approximately 25 ml., and kept. In the course of 3 weeks a small amount of reddish-brown pigment (*C*) (1—1·5 g.) separated, and, after its removal, the liquor was further concentrated to about 10 ml. When kept, this concentrate gradually (about 2 weeks) deposited santal as a reddish crystalline solid; the yield of crude compound was approximately 2 g. from 1800 g. of wood, and a further quantity (0·5 g.) separated from the residues after several months. The crude material was triturated with a little cold alcohol, dried, and then purified by recrystallisation from alcohol or ethyl acetate, giving santal, as a monohydrate, in lustrous yellow plates (1·2 g. from 2 g. of crude product), m. p. 223°, unchanged on sublimation at 190—200°/0·0005 mm. [Found (in specimen dried in air or in a vacuum-desiccator at room temperature): C, 60·5; H, 4·5; OMe, 8·8; $C_{15}H_9O_5(OMe),H_2O$ requires C, 60·4; H, 4·4; OMe, 9·8%. Found (in material sublimed in a high vacuum): C, 63·9; H, 4·0; OMe, 9·7. Calc. for $C_{15}H_9O_5(OMe)$: C, 63·9; H, 4·0; OMe, 10·3%]. Santal gives an intense brownish-red ferric reaction in alcohol. When kept, colourless aqueous alkaline solutions of the compound slowly became green. Acetylation of santal (0·5 g.) with boiling acetic anhydride (10 ml.) and sodium acetate (0·5 g.) gave rise to *O-triacetylsantal* (0·6 g.) which separated from methanol or acetic acid in colourless needles, m. p. 170°, after sintering at 152°, having a negative ferric reaction (Found: C, 62·4; H, 4·4. $C_{22}H_{18}O_8$ requires C, 62·0; H, 4·2%). These analytical results are in close agreement with those given by Raudnitz and Perlmann for their so-called diacetyl derivative.

O-Trimethylsantal (II; R = Me).*—Santal (1 g.) was methylated with methyl iodide (2 ml.) and potassium carbonate (10 g.) in boiling acetone (75 ml.) during 12 hours, with the addition of further portions of iodide (1·5 g.) and carbonate (3 g.) after 4 hours and then after 8 hours. After isolation from the acetone liquor, *O-trimethylsantal* crystallised from warm alcohol in colourless needles (0·9 g.), m. p. 155—156°, which after repeated purification from alcohol or ethyl acetate had m. p. 166° [Found: C, 66·5; H, 5·5; OMe, 33·5. $C_{15}H_8O_2(OMe)_4$ requires C, 66·7; H, 5·3; OMe, 36·3%]. It seems hardly likely that this ether is the same compound as the so-called dimethyl ether, m. p. 142°, described by Raudnitz and Perlmann (*loc. cit.*). *O*-Trimethylsantal which has a negative ferric reaction and does not react with 2 : 4-dinitrophenylhydrazine, is readily soluble in acetone, moderately soluble in benzene, and insoluble in light petroleum or aqueous sodium hydroxide. On being warmed the deep green solution of the ether in concentrated sulphuric acid becomes yellow.

The hydrogenation of *O*-trimethylsantal (0·9 g.) in acetic acid (110 ml.) at 100° with a palladium–charcoal catalyst (from 2 g. of charcoal and 0·2 g. of palladium chloride) and hydrogen at atmospheric pressure appeared to be complete in about 25 minutes. The resulting 5 : 7 : 3′ : 4′-*tetramethoxyisoflavan* (IX) was crystallised from alcohol, forming colourless silky needles (0·55 g.), m. p. 134°, insoluble in aqueous sodium hydroxide and having a negative ferric reaction [Found: C, 68·7; H, 6·6; OMe, 33·9. $C_{15}H_{10}O(OMe)_4$ requires C, 69·1; H, 6·7; OMe, 37·6. $C_{15}H_8O_2(OMe)_4$ requires C, 66·3; H, 5·8; OMe, 36·0%]. This substance, which is readily soluble in acetone, ethyl acetate, and benzene, and insoluble in light petroleum, gives a pale yellow solution in sulphuric acid which on being warmed becomes green and then ruby-red. For an unknown reason it was not always possible to effect this hydrogenation, and then only unchanged *O*-trimethylsantal was obtained.

A saturated solution of potassium permanganate (100 ml.) was added dropwise during 3 hours to the foregoing *iso*flavan (1 g.), dissolved in acetone (150 ml.), and next day the mixture was cleared with sulphur dioxide, warmed with a little 2N-sulphuric acid, cooled, and diluted with water. Unchanged compound (0·6 g.), m. p. 133°, quickly separated, and, after the isolation of this substance and spontaneous evaporation of the acetone, the liquor slowly (2 weeks) deposited a product (0·1 g.), m. p. 151—156°, which is probably 5 : 7 : 3′ : 4′-tetramethoxy*iso*flavanone. Recrystallised from alcohol or a little ethyl acetate, this compound formed white needles, m. p. 158·5°, which had a negative ferric reaction and

* A small specimen of santal, m. p. 218°, was isolated by H. W. Jones in collaboration with one of us (A. R.) during 1939—1940 from an ethereal extract of sandelwood which had been exhausted with carbon tetrachloride. This material gave analytical figures agreeing reasonably well with those now obtained for the monohydrate of santal (Found: C, 60·2; H, 4·6; OMe, 11·5%), and formed the trimethyl ether, m. p. 166° (Found: OMe, 35·8%) and an acetyl derivative (? *O*-diacetate), m. p. 152°. These results were embodied in a Thesis submitted by H. W. Jones for the degree of Master of Science of the University of Liverpool in May, 1941.—A. R.

readily reacted with 2 : 4-dinitrophenylhydrazine. Attempts to improve the yield of this *iso*flavanone by varying the conditions of oxidation, *e.g.*, elevated temperatures or presence of potassium carbonate, failed.

Ozonolysis of O-*Trimethylsantal.*—Ozone and oxygen were passed for ½ hour into a solution of the ether (1 g.) in chloroform (100 ml.) at 0°, and the solvent was then removed in a vacuum. On treatment with water the combined brown oily products from two experiments set to a hard glass which was disintegrated by being triturated with 2N-sodium hydroxide. The insoluble material (0·09 g.), consisting of unchanged *O*-trimethylsantal, was separated by filtration, and the filtrate acidified with hydrochloric acid and extracted with ether. Crystallised from alcohol and then ethyl acetate, the residue left on evaporation of the ethereal extracts gave 2-hydroxy-4 : 6-dimethoxybenzoic acid in colourless needles (0·4 g.), m. p. 156°, identical in every way with an authentic sample prepared according to Herzig *et al.* (*Monatsh.*, 1902, **23**, 95) (Found : C, 54·8; H, 5·2. Calc. for $C_9H_{10}O_5$; C, 54·5; H, 5·5%). Prepared by means of diazomethane, the methyl ester of this acid from either source had m. p. and mixed m. p. 109°. Evaporation of the alcoholic liquors from the purification of 2-hydroxy-4 : 6-dimethoxybenzoic acid left a semi-solid mass, part of which did not dissolve on being triturated with aqueous sodium hydrogen carbonate. Recrystallised from a little alcohol, this non-acidic compound formed colourless needles (0·2 g.), m. p. 77—78°, giving an amethyst colour with alcoholic ferric chloride (Found : C, 58·2; H, 6·1; OMe, 40·9%).

Hydrolysis of O-*Trimethylsantal.*—A solution of this ether (1 g.) in alcohol (47·5 ml.), containing potassium hydroxide (2·5 g.) and water (2·5 ml.), was boiled under reflux for 1 hour, cooled, diluted with water (150 ml.), and acidified (Congo-red) with 2N-hydrochloric acid. The resulting precipitate (0·8 g.) was dried and crystallised from alcohol, giving 2-*hydroxy*-4 : 6 : 3′ : 4′-*tetramethoxydeoxybenzoin* (III) in white needles, m. p. 117°, which had a reddish-brown ferric reaction in alcohol and were soluble in cold 2N-sodium hydroxide [Found : C, 64·9; H, 6·2; OMe, 35·4. $C_{14}H_8O_3(OMe)_4$ requires C, 65·1; H, 6·0; OMe, 37·3%]. The compound is readily soluble in acetone, chloroform, or ethyl acetate, and moderately soluble in benzene or ether.

Hydrolysis of *O*-trimethylsantal (2 g.) with 5% boiling potassium hydroxide (100 ml.) for 1 hour and acidification, after cooling, with 2N-sulphuric acid, yielded a slightly sticky precipitate. Crystallised from alcohol, this gave 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin, m. p. and mixed m. p. 117°. Distillation of the aqueous liquor and subsequent neutralisation of the distillate, followed by evaporation to dryness and heating the residue with aniline (5 ml.) and aniline hydrochloride (2 g.), gave rise to *NN′*-diphenylformamidine hydrochloride (0·2 g.), identified by comparison with authentic material.

Oximation of 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (0·5 g.) in methanol (20 ml.) and water (6 ml.) was effected with hydroxylamine hydrochloride (0·5 g.) and sodium acetate (1·0 g.) on the steam-bath for 8 hours; shorter reaction periods gave unchanged ketone. After the addition of water (25 ml.) to the cooled mixture the *oxime* slowly separated in white needles (0·5 g.), m. p. 134—135°, and on being recrystallised from alcohol or ethyl acetate formed colourless prisms, m. p. 150° (Found : N, 3·9. $C_{18}H_{21}O_6N$ requires N, 4·0%).

Methylation of 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (1 g.) with methyl iodide (3 ml.) and potassium carbonate (10 g.) in boiling acetone (100 ml.) during 8 hours, with the addition of more iodide (3 ml.) and carbonate (5 g.) after 3 hours, gave rise to 2 : 4 : 6 : 3′ : 4′-pentamethoxydeoxybenzoin (0·8 g.), m. p. 97—99°, which on purification from alcohol formed white needles, m. p. 110°, having a negative ferric reaction (Found : C, 65·8; H, 6·2. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4%). This compound was identical with a specimen prepared according to Freudenberg *et al.* (*Annalen*, 1925, **446**, 87).

When potassium permanganate (1·25 g.) in water (50 ml.) was gradually added to 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (0·5 g.) in acetone (75 ml.), a slow oxidation ensued which became somewhat more rapid after a time. Next day the solutions from three experiments were combined, cleared with sulphur dioxide, saturated with ammonium sulphate, and extracted with ether (50 ml. × 5). The residue left on evaporation of the dried extracts was redissolved in ether (100 ml.) and agitated with four successive portions of 2N-sodium hydrogen carbonate (20 ml., each). Acidification of the combined sodium hydrogen carbonate extracts gave veratric acid which was isolated with ether and purified by crystallisation from warm water and then alcohol, forming needles (0·15 g.), m. p. and mixed m. p. 180°, identified by comparison with an authentic sample [Found : C, 59·6; H, 5·7; OMe, 33·2. Calc. for $C_7H_4O_2(OMe)_2$: C, 59·3; H, 5·5; OMe, 34·1%]. The p-*nitrobenzyl* ester was prepared by heating under reflux a mixture of the sodium salt of veratric acid (0·3 g.) in a little water, and *p*-nitrobenzyl bromide (0·1 g.) in alcohol (5 ml.) for 45 minutes. On cooling the mixture, the precipitated ester (0·5 g.) was collected and crystallised from alcohol, forming silky needles, m. p. 144° (Found : N, 4·4. $C_{16}H_{15}O_6N$ requires N, 4·4%), identical with an authentic specimen, m. p. 144°, prepared in the same way (Found : N, 4·4%).

Evaporation of the washed and dried ethereal liquors remaining after the separation of veratric acid gave a product which crystallised from alcohol in pale yellow rhombic prisms (0·8 g.), m. p. 160·5°, having a reddish-brown ferric reaction in alcohol and dissolving in cold 2N-sodium hydroxide [Found : C, 62·2; H, 5·3; OMe, 35·7; *M* (Rast), 331, 303. $C_{14}H_8O_3(OMe)_4$ requires C, 62·1; H, 5·7; OMe, 35·7%; *M*, 348]. This substance, which has solubilities similar to those of the parent ketone and was also formed in poor yield when *O*-trimethylsantal was oxidised by means of potassium permanganate, gave a 2 : 4-*dinitrophenylhydrazone* forming red prisms, m. p. 206°, from ethyl acetate [Found : C, 54·7; H, 4·5; N, 11·2; OMe, 22·6. $C_{20}H_{12}O_6N_4(OMe)_4$ requires C, 54·5; H, 4·5; N, 10·6; OMe, 23·5%]. Methylation of this oxidation product (0·7 g.) by the procedure employed for the parent ketone gave rise to a *monomethyl ether* (*D*) which formed large yellow rhombic tablets (0·6 g.), m. p. 174·5°, insoluble in aqueous sodium hydroxide and having a negative ferric reaction [Found : C, 62·9; H, 6·3; OMe, 43·7. $C_{14}H_7O_2(OMe)_5$ requires C, 63·0; H, 6·1; OMe, 42·8%]. The same ether was obtained when 2 : 4 : 6 : 3′ : 4′-pentamethoxydeoxybenzoin (0·5 g.) in boiling acetone (60 ml.) was oxidised with aqueous potassium permanganate (15 ml. of a solution of 1·25 g. of permanganate in 60 ml. of water) and the solution subsequently cleared with sulphur dioxide and extracted with ether. Crystallised from aqueous alcohol and then from ethyl acetate, this specimen had m. p. and mixed m. p. 174—175°. From the

alcoholic liquors unchanged ketone (0·3 g.) was obtained by evaporation and purified by repeated crystallisation; m. p. 110°.

Interaction of the ether (*D*), with phenylhydrazine under the usual conditions employed for the preparation of phenylhydrazones gave rise to a product which separated from alcohol in pale-yellow hexagonal plates, m. p. 201°, the analytical results for which do not agree with those required for the expected hydrazone $C_{25}H_{28}O_6N_2$ (Found: C, 68·3, 68·4; H, 5·9, 5·8; N, 6·8. Calc. for $C_{25}H_{28}O_6N_2$: C, 66·4; H, 6·2; N, 6·2. Calc. for $C_{21}H_{26}O_6N_2$: C, 69·1; H, 6·0; N, 6·5%).

5 : 7 : 3′ : 4′-*Tetramethoxy-2-methylisoflavone* (IV).—Vigorous acetylation of 2-hydroxy-4 : 5 : 3′ : 4′-tetramethoxydeoxybenzoin (1·5 g.) with boiling acetic anhydride (10 ml.) and sodium acetate (1 g.) for 33 hours gave the *2-methylisoflavone* (1 g.) which formed colourless needles, m. p. 215—216°, from alcohol or ethyl acetate [Found: C, 67·2; H, 5·6; OMe, 30·7. $C_{16}H_8O_2(OMe)_4$ requires C, 67·4; H, 5·6; OMe, 34·8%].

O-*Triethylsantal* (II; R = Et).—A mixture of santal (2 g.), acetone (150 ml.), ethyl iodide (5 ml.), and potassium carbonate (8 g.) was heated under reflux for 24 hours with the addition of further portions of iodide (2 ml. each time) and carbonate (2 g. each time) at intervals of 8, 12, and 18 hours. The *triethyl ether* crystallised from alcohol and then ethyl acetate in colourless needles (2·1 g.), m. p. 111—112°, insoluble in cold aqueous sodium hydroxide, having a negative ferric reaction in alcohol (Found: C, 68·9; H, 6·5. $C_{22}H_{24}O_6$ requires C, 68·8; H, 6·3%), and readily soluble in acetone and moderately soluble in benzene.

O-Triethylsantal (1 g.) was boiled under reflux in a solution of potassium hydroxide (25 g.) in water (25 ml.) and alcohol (25 ml.) for 1·5 hours, and after the addition of water (15 ml.) the cooled solution was acidified with 2N-hydrochloric acid. Next day the precipitate was collected, washed, dried, and crystallised from alcohol and then from a little ethyl acetate, giving *2-hydroxy-4-methoxy-6 : 3′ : 4′-triethoxydeoxybenzoin* (VII) in colourless hexagonal prisms (0·65 g.), m. p. 99°, soluble in acetone, chloroform, or in cold aqueous sodium hydroxide, and having a brownish-red ferric reaction in alcohol (Found: C, 67·5; H, 7·0. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0%). The *oxime* separated from aqueous alcohol in colourless needles, m. p. 138° (Found: N, 3·5. $C_{21}H_{27}O_6N$ requires N, 3·6%). Oxidation of the ketone (0·6 g.) in acetone (75 ml.) was effected with a solution of potassium permanganate (1·5 g.) in water (50 ml.), added drop-wise, and next day the reaction mixture was cleared with sulphur dioxide, boiled with a little dilute sulphuric acid for two minutes, cooled, saturated with ammonium sulphate, and extracted with ether (50 ml. × 5). The combined extracts were evaporated, and a solution of the residue in ether was extracted with 2N-sodium hydrogen carbonate (20 ml. × 4). Acidification of the combined carbonate washings gave 3 : 4-diethoxybenzoic acid (0·3 g.), m. p. 156—163°, which on recrystallisation from aqueous acetone or alcohol had m. p. 165—166°, undepressed on admixture with an authentic specimen prepared by the oxidation of 3 : 4-diethoxybenzaldehyde (Herzig, *Monatsh.*, 1884, **5**, 78) [Found: C, 62·7; H, 6·8; equiv. (by titration), 216. $C_{11}H_{14}O_4$ requires C, 62·9; H, 6·7%; equiv., 210]. A specimen of the p-*nitrobenzyl* ester of the acid from natural sources was prepared by the method employed in the case of veratric acid and formed silky needles, m. p. 100°, from alcohol (Found: N, 4·0. $C_{18}H_{19}O_6N$ requires N, 4·1%); it was identified by comparison with a specimen, m. p. 100°, prepared from an authentic sample of 3 : 4-diethoxybenzoic acid (Found: N, 4·3%).

The ethereal solution left after the separation of 3 : 4-diethoxybenzoic acid was dried and evaporated; the residue, on crystallisation from alcohol and then from ethyl acetate–light petroleum (b. p. 60—80°) gave a *compound* (1·2 g.) in needles, m. p. 114—115°, slowly soluble in cooled 2N-sodium hydroxide and having a reddish-brown ferric reaction in alcohol (Found: C, 64·9; H, 6·2. $C_{21}H_{26}O_7$ requires C, 64·6; H, 6·7%). A solution of this substance in concentrated sulphuric acid had a deep puce colour, unchanged on gentle warming, whilst the blue solution in concentrated nitric acid changed to scarlet on being gently warmed.

2 : 4-*Dihydroxy*-6 : 3′ : 4′-*trimethoxydeoxybenzoin* (V; R = R′ = Me).—A mixture of phloroglucinol monomethyl ether (5 g.), powdered zinc chloride (2 g.), and 3 : 4-dimethoxybenzyl cyanide (6 g.) in ether (200 ml.) was saturated with hydrogen chloride in the course of 3 hours. Next day the crystalline ketimine derivative was well washed with ether and hydrolysed with water (100 ml.) on the steam-bath for ¾ hour, giving 2 : 4-*dihydroxy*-6 : 3′ : 4′-*trimethoxydeoxybenzoin*, which separated from the cooled hydrolysate. Recrystallised from alcohol, this ketone formed minute rhombic prisms (2·2 g.), m. p. 180° (Found: C, 63·8; H, 5·7. $C_{17}H_{16}O_6$ requires C, 64·2; H, 5·7%).

2-*Hydroxy*-4 : 6 : 3′ : 4′-*tetramethoxydeoxybenzoin* (III).—(*a*) Prepared from a mixture of *O*-dimethylphloroglucinol (6 g.), 3 : 4-dimethoxybenzyl cyanide (6 g.), and zinc chloride (2 g.) in ether (100 ml.) saturated with hydrogen chloride, the oily ketimine derivative was well washed with ether and dissolved in water (100 ml.). After having been almost neutralised with aqueous sodium hydrogen carbonate, this solution was heated on the steam-bath for 90 minutes, and the product which separated from the cooled mixture was collected, washed, and recrystallised several times from alcohol, giving 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin in characteristic rosettes of colourless needles (1·5 g.), m. p. 117°, identical with the ketone obtained by the alkaline hydrolysis of *O*-trimethylsantal (Found: C, 65·0; H, 6·3%). Concentration of the alcoholic liquors from the purification of this ketone gave 4-*hydroxy*-2 : 6 : 3′ : 4′-*tetramethoxydeoxybenzoin* (VI) which separated from dilute alcohol in colourless prisms (0·3 g.), m. p. 140°, readily soluble in dilute aqueous sodium hydroxide and having a negative ferric reaction in alcohol (Found: C, 64·8; H, 6·2%). Alternatively, the ketones were separated as follows: a chloroform extract of the hydrolysate was extracted with 1% aqueous sodium hydroxide, and the chloroform solution washed, dried, and evaporated, leaving (III), m. p. 117° (after purification). Acidification of the alkaline extract gave the isomeric 4-hydroxy-ketone, m. p. 140°.

(*b*) A solution of 2 : 4-dihydroxy-6 : 3′ : 4′-trimethoxydeoxybenzoin (1·8 g.) in acetone (50 ml.) containing potassium carbonate (5 g.) and methyl iodide (2 ml.) was boiled for 1 hour; the product was crystallised from alcohol, giving 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxyphenacetophenone in colourless needles (1·5 g.), m. p. and mixed m. p. 117°.

Methylation of 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (2 g.) by potassium carbonate (15 g.) and methyl iodide (15 ml.) in boiling acetone during 11 hours gave 2 : 4 : 6 : 3′ : 4′-pentamethoxy-

deoxybenzoin (1·8 g.), m. p. 109—110°, undepressed on admixture of a specimen prepared by the method of Freudenberg *et al.* (*Annalen*, 1924, **446**, 87) (Found: C, 65·6; H, 6·4. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%).

Oxidation of synthetic 2-hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin by the method employed for the compound obtained from *O*-trimethylsantal gave the same product, m. p. and mixed m. p. 160·5°.

5 : 7 : 3′ : 4′-*Tetramethoxyisoflavone* (II; R = Me).—2-Hydroxy-4 : 6 : 3′ : 4′-tetramethoxydeoxybenzoin (1 g.), ethyl formate (1·5 ml.), and pulverised sodium (1 g.) were mixed at −10° and then kept at −5° for 2 days. After addition of ice the mixture was extracted with ether (50 ml. × 4), and the combined extracts were washed, dried, and evaporated, leaving the *iso*flavone as an oil which, on crystallisation from alcohol and then from ethyl acetate, formed colourless needles, m. p. 164—166°, identical with *O*-trimethylsantal (Found: C, 66·9; H, 5·5%).

2 : 4-*Dihydroxy*-6 : 3′ : 4′-*triethoxydeoxybenzoin* (V; R = Et).—The condensation of phloroglucinol monoethyl ether (4 g.) and 3 : 4-diethoxybenzyl cyanide (4 g.) in ether (20 ml.) by means of zinc chloride (2 g.) and excess of hydrogen chloride gave a brown viscous oil, which was well washed with ether and dissolved in water (100 ml.). This solution was almost neutralised with saturated aqueous sodium hydrogen carbonate and then heated on the steam-bath for 90 minutes. After the mixture cooled, the precipitated brownish oily product gradually solidified, and on crystallisation from alcohol and then ethyl acetate gave the 2 : 4-*dihydroxy*-6 : 3′ : 4′-*triethoxydeoxybenzoin* (V; R = R′ = Et) in spherical clusters of rhombic prisms (1·1 g.) which had a reddish-brown ferric reaction in alcohol (Found: C, 66·7; H, 6·8. $C_{20}H_{24}O_6$ requires C, 66·7; 6·7%). This substance is readily soluble in acetone, moderately soluble in benzene, and almost insoluble in light petroleum.

Partial methylation of this ketone (1·2 g.) was effected with methyl iodide (0·8 g.) and potassium carbonate (2 g.) in boiling acetone (50 ml.) during 1 hour with the addition of more iodide (0·4 ml.) after 30 minutes. The product was crystallised from 95% alcohol, giving 2-hydroxy-4-methoxy-6 : 3′ : 4′-triethoxydeoxybenzoin (VII) in colourless needles, m. p. 99°, identical in every way with a specimen obtained by the hydrolytic fission of *O*-triethylsantal (Found: C, 65·4; H, 7·2%).

7-*Methoxy*-5 : 3′ : 4′-*triethoxy*isoflavone (O-*Triethylsantal*) (II; R = Et).—The condensation of methyl formate (25 ml.) and 2-hydroxy-4-methoxy-6 : 3′ : 4′-triethoxydeoxybenzoin (0·7 g.) by means of sodium (0·7 g.) at −15° and then at −5° for 3 days gave an oily product which did not solidify and which may have been the formyl derivative of the ketone. A solution of this in acetic acid (5 ml.) was heated under reflux for 30 minutes, diluted with water (30 ml.), neutralised with sodium hydrogen carbonate, and extracted with ether (30 ml. × 4). Crystallised from ethyl acetate, the residue obtained on evaporation of the dried extracts gave the *iso*flavone (0·5 g.) in colourless needles, m. p. 111—112°, identical with a specimen of *O*-triethylsantal (Found: C, 68·6; H, 6·1%).

2 : 4-*Dihydroxy*-6-*methoxy*-3′ : 4′-*diethoxydeoxybenzoin* (V; R = Me; R′ = Et).—From the interaction of phloroglucinol monomethyl ether (4 g.) and 3 : 4-diethoxybenzyl cyanide (4 g.), zinc chloride (2 g.), and excess of hydrogen chloride in ether (200 ml.) during 4 days, a viscous olive-brown product was obtained which was dissolved in water (200 ml.). After having been almost neutralised with sodium hydrogen carbonate and then heated on the steam-bath for 90 minutes, this solution gave 2 : 4-*dihydroxy*-6-*methoxy*-3′ : 4′-*diethoxydeoxybenzoin* as a brown oil which slowly solidified and separated from alcohol and then ethyl acetate in elongated rhombic tablets (1·6 g.), m. p. 129—130°, having a reddish-brown ferric reaction in alcohol (Found: C, 65·9; H, 6·5. $C_{19}H_{22}O_6$ requires C, 65·9; H, 6·4%). Recrystallisation of the pure ketone from 95% alcohol gave material of a slightly lower and somewhat variable melting point, a result apparently due to solvation since on being dried in a high vacuum at 80° this product then melted at 129—130°. The compound is readily soluble in dilute aqueous sodium hydroxide or acetone, and sparingly soluble in benzene or ether.

Monoethylation of this ketone (1·2 g.) with ethyl iodide (0·6 ml.) and potassium carbonate (1·5 g.) in boiling acetone (22 ml.) during 2 hours gave 2-*hydroxy*-4 : 6 : 3′ : 4′-*tetraethoxydeoxybenzoin* which formed colourless needles (1·0 g.), m. p. 111—112°, from alcohol or ethyl acetate, having a reddish-brown ferric reaction in alcohol (Found: C, 67·2; H, 6·8. $C_{21}H_{26}O_6$ required C, 67·4; H, 7·0%). This ketone is slowly soluble in 2N-sodium hydroxide and, on being warmed, its pale green solution in concentrated sulphuric acid becomes lilac and then brown, whilst the green solution of the substance in concentrated nitric acid quickly becomes blue and then orange.

5-*Methoxy*-7 : 3′ : 4′-*triethoxy*isoflavone (II; R = Et).—2-Hydroxy-6-methoxy-4 : 3′ : 4′-triethoxydeoxybenzoin (0·7 g.), methyl formate (15 ml.), and pulverised sodium were mixed at −10° and then kept at −5° for 3 days. After the addition of ice the *isoflavone* was isolated by repeated extraction with ether and then crystallised from ethyl acetate, forming thin parallelepipeds (0·4 g.), m. p. 129—130°, readily soluble in acetone and moderately soluble in alcohol (Found: C, 69·0; H, 6·4. $C_{23}H_{24}O_6$ requires C, 68·8; H, 6·3%).

2-*Hydroxy*-3′-*methoxy*-4 : 6 : 4′-*triethoxydeoxybenzoin*.—The reddish oil obtained from the interaction of phloroglucinol diethyl ether (4 g.), 3-methoxy-4-ethoxybenzyl cyanide (4 g.), zinc chloride (2 g.), and excess of hydrogen chloride in ether (150 ml.) was dissolved in water (200 ml.), and after having been almost neutralised with sodium hydrogen carbonate the solution was heated on the steam-bath for 1½ hours. A solution of the resulting oily product in chloroform was washed once with water and then extracted with 1% aqueous sodium hydroxide (20 ml. × 5), dried, and evaporated. Crystallisation of the residue from a little alcohol gave 2-*hydroxy*-3′-*methoxy*-4 : 6 : 4′-*triethoxydeoxybenzoin* (1·25 g.) which on recrystallisation from alcohol or ethyl acetate formed colourless needles, m. p. 138°, slowly soluble in 2N-sodium hydroxide and having a reddish-brown ferric reaction in alcohol (Found: C, 67·2; H, 7·3. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0%). Acidification of the 1% aqueous sodium hydroxide extracts gave a small amount of an oil which did not solidify and may have contained the isomeric 4-hydroxyketone along with unchanged phloroglucinol diethyl ether.

3′-*Methoxy*-5 : 7 : 4′-*triethoxy*isoflavone.—Condensation of the foregoing ketone (1·2 g.) with methyl formate (50 ml.) by means of sodium (1·2 g.) at −5° for 3 days gave rise to the iso*flavone* (0·4 g.) which on repeated crystallisation from ethyl acetate was obtained in colourless needles, m. p. 134°, changing

to 150° after having been dried in a high vacuum at 80° for 2 hours [Found (in dried material): C, 68·9; H, 6·4. $C_{22}H_{24}O_6$ requires C, 68·8; H, 6·3%].

4'-Methoxy-5 : 7 : 3'-triethoxyisoflavone.—The crude product formed by the condensation of phloroglucinol diethyl ether (3·1 g.) and 4-methoxy-3-ethoxybenzyl cyanide (3·1 g.) according to the method of Hoesch was dissolved in chloroform, and the solution extracted with 1% aqueous sodium hydroxide, dried, and evaporated, leaving *2-hydroxy-5'-methoxy-4 : 6 : 3'-triethoxydeoxybenzoin* (1 g.) which formed colourless rectangular tablets, m. p. 117°, from alcohol or ethyl acetate (Found: C, 67·4; H, 7·0. $C_{21}H_{26}O_6$ requires C, 67·4; H, 7·0%). This compound has a reddish-brown ferric reaction in alcohol, and on being warmed the pale green solution in concentrated sulphuric acid becomes deep green and then amber. From this ketone *4'-methoxy-5 : 7 : 3'-triethoxyisoflavone* was prepared in the same manner as 3'-methoxy-5 : 7 : 4'-triethoxyisoflavone and on crystallisation from alcohol formed colourless needles, m. p. 114° [Found (in specimen dried in a high vacuum at 80°): C, 68·7; H, 6·1. $C_{22}H_{24}O_6$ requires C, 68·8; H, 6·3%].

1-3' : 4'-Dimethoxyphenyl-2-2'' : 4'' : 6''-trimethoxybenzoylethylene oxide was prepared in the course of attempts to define the oxidation product of 2-hydroxy-4 : 6 : 3' : 4'-tetramethoxyphenacetophenone. A solution of 2 : 4 : 6-trimethoxyacetophenone (4 g.) and veratraldehyde (4 g.) in the minimum amount of alcohol was mixed with aqueous sodium hydroxide (8 g. in 16 ml. of water), boiled under reflux for 1 hour, and poured on ice. The resulting styryl ketone separated from ethyl acetate in yellow prisms (5·5 g.), m. p. 117° (compare Tutin and Caton, *J.*, 1910, **97**, 2062). It (5·8 g.) was oxidised in methanol (30 ml.) at 25° with a mixture of 15% hydrogen peroxide (5 ml.) and 2N-sodium hydroxide during 2 hours, and the clear solution then diluted with water. Crystallised from ethyl acetate–light petroleum (b. p. 60—80°) and then from alcohol or ethyl acetate, the resulting precipitate gave the oxide in colourless silky needles (4·5 g.), m. p. 117—118°, readily soluble in acetone (Found: C, 64·2; H, 6·0. $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%).

2-Hydroxy-4 : 6-dimethoxy- and *2-Hydroxy-4 : 6 : 4'-trimethoxy-deoxybenzoin.*—The condensation of benzyl cyanide (5 g.) and phloroglucinol dimethyl ether (5 g.) by the method of Hoesch gave an oily product which was dissolved in chloroform; the solution was then extracted with 1% aqueous sodium hydroxide to remove unchanged phloroglucinol dimethyl ether and any 4-hydroxy-2 : 6-dimethoxydeoxybenzoin. Evaporation of the chloroform solution left the required *2-hydroxy-4 : 6-dimethoxydeoxybenzoin*, which separated from 95% alcohol and then ethyl acetate in large aggregates of colourless rhombic plates (2·5 g.), m. p. 117°, having a reddish-brown ferric reaction in alcohol (Found: C, 70·3; H, 6·0. $C_{16}H_{16}O_4$ requires C, 70·6; H, 5·9%).

Potassium permanganate (2·5 g.), dissolved in water (100 ml.), was added gradually in 20-ml. portions to a solution of 2-hydroxy-4 : 6-dimethoxydeoxybenzoin (1 g.) in acetone (75 ml.). Next day the mixture was cleared with sulphur dioxide; in the course of 24 hours a *product* (0·8 g.) separated in silky needles, insoluble in aqueous sodium hydrogen carbonate but readily soluble in aqueous sodium hydroxide. Recrystallised from ethyl acetate–light petroleum (b. p. 80—100°), the compound had m. p. 110—111° and on admixture with the parent ketone melted at 98° (Found: C, 66·7; H, 5·5. $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6%); it is slowly soluble in aqueous sodium carbonate, and gives a reddish-brown ferric reaction in alcohol. Benzoic acid appears not to have been formed in appreciable quantity in this oxidation.

In a similar manner *2-hydroxy-4 : 6 : 4'-trimethoxydeoxybenzoin* (1·64 g.) was prepared from phloroglucinol dimethyl ether (4·5 g.) and 4-methoxybenzyl cyanide (4·5 g.) and on crystallisation from alcohol formed white needles, m. p. 88—89°, slowly soluble in 2N-sodium hydroxide and having a reddish-brown ferric reaction (Found: C, 67·8; H, 6·1. $C_{17}H_{18}O_5$ requires C, 67·6; H, 6·0%). Oxidation of this ketone (1 g.) in acetone (75 ml.) was effected with potassium permanganate (2·5 g.) in water (100 ml.) added in portions of about 20 ml. To initiate the reaction after the addition of the first portion of permanganate solution it was necessary to warm the mixture gently on the steam-bath for a few minutes, but subsequently the mixture was cooled in tap-water before each fresh addition of the reagent. After having been cleared with sulphur dioxide, the reaction mixture was saturated with ammonium sulphate and extracted with ether (30 ml. × 4). The residue left on evaporation of the combined ethereal extracts which had contained acetone was dissolved in fresh ether and then extracted with saturated aqueous sodium hydrogen carbonate (25 ml. × 4). Acidification of the latter combined extracts gave anisic acid (0·75 g.), m. p. and mixed m. p. 183° (after purification). Evaporation of the dried ethereal solution left after the separation of anisic acid gave an oil which was dissolved in a mixture of boiling ethyl acetate and light petroleum (b. p. 80—100°). On cooling this solution deposited a *compound* in pale yellow needles (0·7 g.), m. p. 122°, soluble in cold 2N-sodium carbonate, and having a reddish-brown ferric reaction in alcohol (Found: C, 64·1; H, 5·7. $C_{17}H_{18}O_6$ requires C, 64·2; H, 5·7%).

Demethylation of Santal.—Acetic anhydride (20 ml.) was carefully added to hydriodic acid (5 ml., sp. gr. 1·7) with cooling. Santal (1 g.) was then introduced, and the mixture heated under reflux (oil-bath at 150°) for 30 minutes, cooled, mixed with saturated aqueous sodium sulphite (50 ml.), and saturated with ammonium sulphate. Next day the yellow precipitate (0·8 g.) was collected, well washed with water, and crystallised from 50% acetic acid, giving *5 : 7 : 3' : 4'-tetrahydroxyisoflavone* (norsantal) (I; R = H) in cream-coloured, globular clusters of minute tablets which, on being sublimed at 230°/0·005 mm., formed a mass of pale yellow, elongated, rhombic plates, m. p. 270° (Found: C, 63·1; H, 3·6. Calc. for $C_{15}H_{10}O_6$: C, 63·0; H, 3·5%). Acetylation of this compound (0·3 g.) with boiling acetic anhydride (5 ml.) and sodium acetate (0·2 g.) gave rise to *5 : 7 : 3' : 4'-tetra-acetoxyisoflavone* (0·3 g.) which on recrystallisation from acetic acid formed colourless needles, m. p. 207·5°, unchanged on further purification from alcohol and ethyl acetate (Found: C, 60·5; H, 4·1. Calc. for $C_{23}H_{18}O_{10}$: C, 60·8; H, 4·0%).

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337. *The Symmetrical Dianthryls. Part II.*

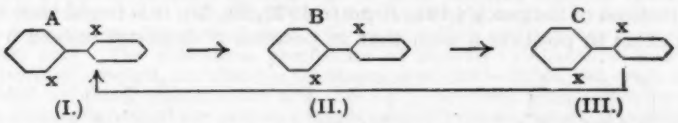
By F. Bell and D. H. Waring.

Optically active 1 : 1′-dianthryl-2 : 2′-dicarboxylic acid (improved preparation and resolution) can be converted into 2 : 2′-diamino-1 : 1′-dianthryl and this back into the initial material by two different routes without change either in magnitude or in sign of rotation.

(+)- and (−)-1 : 1′-Dianthryls are obtained from (+)- and (−)-2 : 2′-diamino-1 : 1′-dianthryls. This hydrocarbon is racemised in hot solvents.

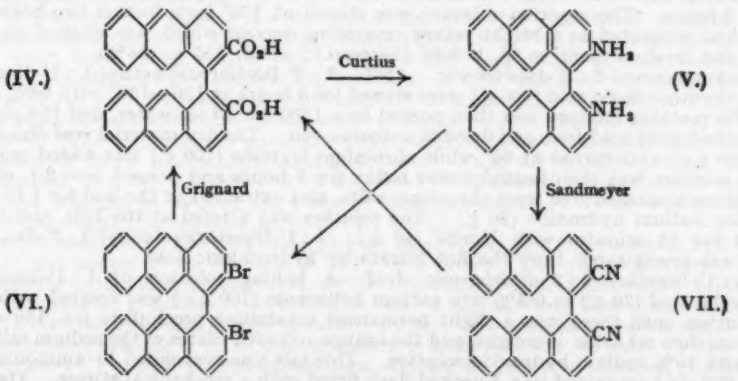
An improved preparation of anthrapinacol allows the ready preparation of 9 : 9′-dianthryl.

The object of the present investigation was to carry out a cycle of the type shown, commencing with one of the optically active forms of (I). The asymmetry of (I) is determined by restricted rotation about the single linkage connecting the nuclei. In the changes I ⟶ II,



II ⟶ III, and III ⟶ I, one of the obstacle groups is removed completely and replaced by a different group. It appeared to the authors impossible, from existing knowledge, to state in advance whether, after such a process, compound (I) would be recovered with undiminished rotatory power and, indeed, even with the same sign of rotation. Two considerations must govern the choice of starting material : (*a*) that it possesses good optical stability, and (*b*) that it lends itself to a cycle in which the intermediates (II) and (III) are likely themselves to be optically stable. 1 : 1′-Dianthryl-2 : 2′-dicarboxylic acid (IV) appeared to fulfil these conditions, as it was known from the work of Lauer, Oda, and Miyawaki (*J. pr. Chem.*, 1937, [ii], **148**, 310) that the optically active acid could be converted by the Hofmann reaction into 2 : 2′-diamino-1 : 1′-dianthryl of high rotatory power.

Scholl and Tanzer (*Annalen*, 1923, **433**, 163) obtained 1 : 1′-dianthryl-2 : 2′-dicarboxylic acid by the reduction of 1 : 1′-dianthraquinonyl-2 : 2′-dicarboxylic acid, and describe it as pale greenish-yellow needles or leaflets which char at about 183° and melt indefinitely at 273—275°. Lauer, Oda, and Miyawaki (*loc. cit.*), using the same method of preparation, obtained greenish-yellow needles, m. p. 201—203° (decomp.). Resolution of the acid into optically active forms was accomplished by a curious and empirical process involving the use of quinine. It was clearly necessary to re-examine both the preparation and the resolution of the acid.



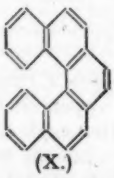
In the Experimental section details are given for the preparation of 1 : 1′-dianthraquinonyl-2 : 2′-dicarboxylic acid in considerable quantity and in much higher purity than is obtained by the oxidation of 2 : 2′-dimethyl-1 : 1′-dianthraquinonyl. This compound gave on reduction 1 : 1′-dianthryl-2 : 2′-dicarboxylic acid of m. p. 305°. The inactive acid was readily resolved by crystallisation of the quinidine salt. The optically active forms, which were noticeably more soluble than the inactive acid, had the following properties : *dextro*, $[\alpha]_D$ +382°, m. p. 251—253°; *lævo*, $[\alpha]_D$ −386°, m. p. 253—255°. Lauer, Oda, and Miyawaki give *dextro*, $[\alpha]_D$ +352°, m. p. 187—198°; *lævo*, $[\alpha]_D$ −358°, m. p. 190—200°. The *dextro*- and *lævo*-acids were converted by the Curtius reaction into *dextro*- and *lævo*-2 : 2′-diamino-1 : 1′-dianthryl (V). The *dextro*-diamine was converted by diazotisation

into 2 : 2′-dibromo-1 : 1′-dianthryl (VI) and this by the Grignard reaction into the original acid, which had $[\alpha]_{5461}^{20°}$ +422°. The *lævo*-diamine was converted by the Sandmeyer reaction into the dicyanide (VII) and this by hydrolysis with potassium hydroxide in ethylene glycol into the original acid which had $[\alpha]_{5461}^{20°}$ −437°. In both cycles, therefore, the acid is recovered unchanged both in sign and, essentially, in magnitude of rotation.

This so conclusive result shows that, if the reactions involve the formation of an ion or radical of type (VIII), this is possessed of a high degree of optical stability. In consequence, 1 : 1′-dianthryl itself might be expected to exist in optically active forms of considerable stability. This was found to be the case. *dextro-* and *lævo*-1 : 1′-Dianthryl were prepared from *dextro-* and *lævo*-2 : 2′-diamino-1 : 1′-dianthryl (V). The optically active forms were completely stable in chloroform at room temperature, but were racemised in about 7 hours at the boiling point or more rapidly in boiling xylene solution. When a drawing of this hydrocarbon is made according to the method of Sidgwick (*Ann. Reports*, 1932, **29**, 70), it is found that the interference of the hydrogen atom in position 9 with that in position 2′ does not exceed 0·2 A.

(VIII.)

(IX.)



(X.)

It appears from this result that compounds such as 1 : 1′-dinaphthyl (IX) and 3 : 4 : 5 : 6-dibenzphenanthrene (X) might be expected to exist in optically active forms (cf. Cook, *Ann. Reports*, 1942, **39**, 173), and these and related matters are under active investigation.

In Part I (this vol., p. 267) methods for preparing 9 : 9′-dianthryl were summarised.* It is now found that the most efficient process is the reduction of anthrone to anthrapinacol and dehydration of the latter by the method of Bergmann and Schuchardt (*Annalen*, 1931, **487**, 225); the overall yield is 50% based on anthraquinone.

Experimental.

(Analyses are by Drs. Weiler and Strauss, Oxford. M. p.s are uncorrected.)

2 : 2′-*Bisdibromomethyl*-1 : 1′-*dianthraquinonyl*.—Crude 2 : 2′-dimethyl-1 : 1′-dianthraquinonyl was purified by crystallisation from *o*-dichlorobenzene. 175 G. of the purified material were stirred in 1 l. of dry nitrobenzene at 145—150° while a mixture of 85 c.c. of bromine in 200 c.c. of dry nitrobenzene was run in during 3 hours. The reaction mixture was stirred at 170° for a further two hours. The tetrabromo-compound separated as a bright yellow crystalline deposit which was washed on the filter with nitrobenzene and finally ether; m. p. >300° (decomp.); yield, 255 g. (84%).

1 : 1′-*Dianthraquinonyl*-2 : 2′-*dicarboxylic Acid*.—2 : 2′-Bisdibromomethyl-1 : 1′-dianthraquinonyl (100 g.) and anhydrous boric acid (100 g.) were stirred for 5 hours at 120—130° with 98% sulphuric acid (600 c.c.). The reaction mixture was then poured into 1500 c.c. of ice-water, and the pink precipitate filtered off, washed until acid-free, and dried in a steam-oven. The dry material was dissolved in glacial acetic acid (600 c.c.) and stirred at 60° while chromium trioxide (100 g.) was added gradually during 1 hour. The mixture was then heated under reflux for 5 hours and poured into 2 l. of water. The product was filtered, washed free from chromium salts, and extracted at the boil for 1 hour with water (3 l.) containing sodium hydroxide (20 g.). The solution was filtered at the boil, and the bright red filtrate boiled for 15 minutes with Norite (10 g.). 1 : 1′-Dianthraquinonyl-2 : 2′-dicarboxylic acid (33 g., 47%) was precipitated from the hot filtrate by hydrochloric acid.

Inactive 1 : 1′-*Dianthryl*-2 : 2′-*dicarboxylic Acid*.—A boiling solution of 1 : 1′-dianthraquinonyl-2 : 2′-dicarboxylic acid (20 g.) in 0·5% w/v sodium hydroxide (700 c.c.) was treated with 25% sodium hydroxide solution until there was a slight permanent crystalline precipitate (*ca.* 150 c.c. required). The solution was then set aside overnight, and the orange-coloured plates of the sodium salt were washed on the filter with 10% sodium hydroxide solution. This salt was suspended in ammonium hydroxide (sp. gr. 0·88; 600 c.c.) contained in a 3-necked flask fitted with a mechanical stirrer. The mixture was warmed gently while stirred, and the air displaced from the flask by a stream of nitrogen. Zinc dust (50 g.) was added gradually during the first ½ hour, and the mixture then gently boiled under reflux for a further 2 hours. The reduction is complete when the colour of the solution (initially bright red) changes to a yellow colour. The mixture was poured into water (3 l.), boiled for ½ hour, and filtered.

1 : 1′-Dianthryl-2 : 2′-dicarboxylic acid, obtained from the filtrate by hydrochloric acid as a gelatinous yellow precipitate (16 g., 90%), was washed free from mineral acid and dried in the steam-oven. The acid (50 g.) from three such experiments was pulverised in a mortar, heated under reflux for ½ hour with glacial acetic acid (600 c.c.), cooled, filtered, and washed with glacial acetic acid. The filter-cake was

* Dr. E. de Barry Barnett asks us to state that the substance, m. p. above 360°, described by him as 9 : 9-dianthryl (Barnett and Matthews, *J.*, 1923, **123**, 380) has not this constitution. It was produced from some unsuspected impurity in the anthrone used in these experiments and cannot be obtained by reduction of pure anthrone.

crystallised from glacial acetic acid (1400 c.c.), which on cooling deposited the dicarboxylic acid as a yellow, fine, hair-like mass. A further crystallisation from acetic acid gave a product (42 g.), m. p. 298—303°. The pure acid, m. p. 305°, can be obtained as yellow plates or prisms from *o*-dichlorobenzene or methyl ethyl ketone.

Resolution.—Results with brucine, morphine, quinine, cinchonine, or cinchonidine were negative or unsatisfactory.

A suspension of the acid (25 g.) in alcohol (1 l.) was heated under reflux on a water-bath. Finely powdered quinidine ($C_{20}H_{24}O_2N_2,2H_2O$; 41 g.) was added slowly. After addition of about 15 g. of the alkaloid the acid was in solution. Addition of the remainder of the alkaloid caused immediate precipitation of a highly crystalline alkaloidal salt. The mixture was kept warm on the water-bath for a further 10 minutes, cooled, and filtered, and the salt well washed with alcohol. Yield, 30·5 g.; $[\alpha]_{5461}^{20°}$ −509° (*c*, 0·51 in chloroform). The combined mother-liquor and washings were distilled on the water-bath until 800 c.c. of alcohol had been removed, and the residual solution set aside overnight. The small quantity (1·73 g.) of amorphous material which separated was filtered off and discarded. The alcohol was then completely removed from the filtrate, and the residual brown honey-like mass decomposed by warming on the water-bath for ½ hour with 1% sodium hydroxide solution (500 c.c.). After filtration from the precipitated alkaloid, the warm yellow solution was poured into 3·5% hydrochloric acid (200 c.c.). The gelatinous precipitate of *dextro*-1 : 1′-dianthryl-2 : 2′-dicarboxylic acid (12·5 g.) was filtered off, washed, and dried in the steam oven at 90—95°. The crude acid was pulverised in a mortar, added to boiling glacial acetic acid (250 c.c.), and freed from a little undissolved material. The solution on cooling deposited the acid as a bright canary-yellow crystalline powder, m. p. 251—253°, $[\alpha]_{5461}^{21°}$ +362° (*c*, 0·7265 in acetone). Two further crystallisations from glacial acetic acid gave the optically pure acid (8·25 g.), m. p. 251—253°, $[\alpha]_{5461}^{20°}$ +439°, $[\alpha]_{5790}^{20°}$ +392°, $[\alpha]_{D}^{21°}$ +382° (*c*, 0·795 in acetone). The crystalline salt of the *lævo*-acid was extracted with boiling alcohol (500 c.c.); the residual salt (27 g.) had $[\alpha]_{5461}^{20°}$ −522° (*c*, 1·03 in chloroform). Decomposition of this by the above method yielded 12·0 g. of acid which, on crystallisation from boiling glacial acetic acid (120 c.c.), yielded 11·1 g. of bright yellow crystalline powder, m. p. 253—255°, $[\alpha]_{5461}^{20°}$ −429° (*c*, 0·80). A further crystallisation from glacial acetic acid yielded 10·4 g. of optically pure acid, m. p. 253—255°, $[\alpha]_{5461}^{20°}$ −440°, $[\alpha]_{5790}^{20°}$ −395°, $[\alpha]_{D}^{21°}$ −386° (*c*, 0·80 in acetone).

Inactive 2 : 2′-*Diamino*-1 : 1′-*dianthryl.*—1 : 1′-Dianthryl-2 : 2′-dicarboxyl chloride, prepared in the usual way from the acid (5 g.) and thionyl chloride (10 c.c.), was dissolved in 50 c.c. of dry sulphur-free benzene. Finely ground sodium azide (4·0 g.) was added and the red solution heated under reflux for 7½ hours. 3N-Sodium hydroxide (25 c.c.) was added to the cooled solution, and boiling continued for a further 2 hours. The solvent was completely removed by steam, and the solid material extracted with 50% v/v hydrochloric acid. The acid filtrate was neutralised with ammonium hydroxide, and the resulting red solid crystallised from a benzene–ethyl alcohol solution (Norite). Several recrystallisations were necessary to free the amine from a red resinous by-product. The 2 : 2′-diamino-1 : 1′-dianthryl crystallises in small colourless plates, m. p. 233°. Yield, 2·9 g. (68%).

lævo-2 : 2′-*Diamino*-1 : 1′-*dianthryl.*—The procedure was as above, except that the thionyl chloride solution of the acid was not warmed, ½ hour at room temperature being sufficient to complete the reaction. There was less formation of resinous by-products, and two crystallisations (Norite with the first) from benzene–ethyl alcohol yielded 3·1 g. of the *lævo*-diamine (72%), m. p. 182→184°, $[\alpha]_{5461}^{20°}$ −415°, $[\alpha]_{D}^{20°}$ −384° (*c*, 0·40 in chloroform).

dextro-2 : 2′-*Diamino*-1 : 1′-*dianthryl.*—By use of the following quantities, the *dextro*-diamine was obtained by the same procedure: Acid (8·0 g.), thionyl chloride (10 c.c.), benzene (50 c.c.), and sodium azide (5 g.). Yield, 5·6 g. (80%); m. p. 185°; $[\alpha]_{5461}^{20°}$ +417°, $[\alpha]_{D}^{20°}$ +387 (*c*, 0·40 in chloroform).

Inactive 2 : 2′-*Dicyano*-1 : 1′-*dianthryl.*—A solution of the inactive diamine (1·5 g.) in 27% hydrochloric acid (10 c.c.) was diazotised by addition of solid sodium nitrite (0·7 g.). The filtered diazo-solution was added to potassium cuprocyanide (0·8 g. of cuprous cyanide in 20 c.c. of 5% potassium cyanide solution). The mixture was warmed on the water-bath for 15 minutes, and the solid filtered off and washed with dilute hydrochloric acid and water. After drying, the product was crystallised twice from alcohol (Norite). Inactive 2 : 2′-*dicyano*-1 : 1′-*dianthryl* crystallises in yellow needles, m. p. 238°. The solution in alcohol has a strong blue fluorescence. Yield, 0·66 g. (42%) (Found: C, 88·6; H, 4·28. $C_{30}H_{16}N_2$ requires C, 88·8; H, 3·9%).

Hydrolysis.—A solution of the dicyanide (0·4 g.) in ethylene glycol–potassium hydroxide solution (10%; 10 c.c.) was heated in an oil-bath at 160° for 2 hours. The yellow solution on pouring into dilute hydrochloric acid yielded quantitatively 1 : 1′-dianthryl-2 : 2′-dicarboxylic acid.

lævo-2 : 2′-*Dicyano*-1 : 1′-*dianthryl.*—Procedure was as above using *lævo*-diamine (2·6 g.), hydrochloric acid (15 c.c.), and sodium nitrite (AnalaR, 1·0 g.); the potassium cuprocyanide solution was prepared from cuprous cyanide (1 g.) in 5% potassium cyanide solution (25 c.c.). Yield, 1·12 g. (41%). lævo-2 : 2′-*Dicyano*-1 : 1′-*dianthryl* crystallises from alcohol in yellow needles, m. p. 162—165°, $[\alpha]_{5461}^{19°}$ −382° (*c*, 0·5 in alcohol).

Hydrolysis.—The hydrolytic procedure described above, at 110—120° for 2 hours, yielded *lævo*-1 : 1′-dianthryl-2 : 2′-dicarboxylic acid, m. p. 254—255°, after one crystallisation from boiling glacial acetic acid; $[\alpha]_{5461}^{20°}$ −437° (*c*, 0·80 in acetone).

Inactive 2 : 2′-*Dibromo*-1 : 1′-*dianthryl.*—The inactive diamine (3·0 g.) was diazotised as described above and added to a solution of cuprous bromide (0·8 g.) in 48% hydrobromic acid (5 c.c.). After the mixture had been heated on the water-bath for 1 hour, the solid was filtered off, washed, dried, and extracted with boiling alcohol in which it is moderately soluble. Recrystallisation from alcohol yielded inactive 2 : 2′-*dibromo*-1 : 1′-*dianthryl* as fine lemon-yellow needles, m. p. 212° (3·2 g., 80%) (Found: C, 65·8; H, 3·26. $C_{28}H_{16}Br_2$ requires C, 65·6; H, 3·0%).

Preparation of 1 : 1′-*Dianthryl*-2 : 2′-*dicarboxylic Acid by the Grignard Reaction.*—The Grignard solution was prepared by treating the above dibromo-compound (3 g.) in anhydrous ether (150 c.c.) with dry pulverised magnesium (1·0 g.). Five drops of freshly distilled ethyl bromide were added, and the mixture was heated under reflux on the steam-bath for 12 hours (procedure of Bachmann and Kloetzel,

J. Org. Chem., 1938, **3**, 59). The separated magnesium complex was dissolved by adding 50 c.c. of dry benzene, and a stream of dry carbon dioxide was passed into the boiling mixture for 2 hours. The cooled solution was warmed with 50 c.c. of water, and the yellowish organic layer extracted three times with dilute ammonium hydroxide solution. The aqueous extracts yielded a gelatinous yellow precipitate (1·3 g., 51%) on acidification. After crystallisation from glacial acetic acid, it had m. p. 298—301° and gave no depression with 1 : 1′-dianthryl-2 : 2′-dicarboxylic acid.

dextro-2 : 2′-*Dibromo*-1 : 1′-*dianthryl*.—By a similar treatment to the above, the *dextro*-diamine (3·5 g.) was converted into *dextro*-2 : 2′-dibromo-1 : 1′-dianthryl, fine yellow needles, m. p. 168—170° (3·0 g.), $[\alpha]_{5461}^{20°}$ +299° (*c*, 0·5 in alcohol).

Preparation of dextro-1 : 1′-*Dianthryl*-2 : 2′-*dicarboxylic Acid by the Grignard Reaction*.—The procedure was as above, except that less solvent was required owing to the higher solubility of the *dextro*-dibromodianthryl. Anhydrous ether (80 c.c.) and anhydrous benzene (30 c.c.) were used. The crude acid (1·7 g.), after crystallisation from glacial acetic acid, had m. p. 252—253°, $[\alpha]_{5461}^{20°}$ +422° (*c*, 0·80 in acetone).

Deamination of Inactive 2 : 2′-*Diamino*-1 : 1′-*dianthryl*.—A solution of the inactive diamine (3·5 g.) in a mixture of dry benzene (5 c.c.), and ethyl alcohol (20 c.c.) was treated with concentrated sulphuric acid (2 c.c.). To the fine resultant suspension was added, at room temperature, sodium nitrite (1 g.) in water (5 c.c.). To the clear diazo-solution was added copper-bronze (0·5 g.) in small portions. There was a vigorous reaction, and a highly crystalline deposit immediately separated; this was filtered off after the mixture had been heated on the water-bath for ½ hour. The residue after drying was warmed with toluene (10 c.c.) in which only part was soluble. The filtrate on cooling deposited 0·63 g. of small colourless plates, m. p. 318—319° alone or mixed with authentic 1 : 1′-dianthryl. The residue from the toluene extraction was crystallised from a large volume of glacial acetic acid and gave 2 : 2′-*dihydroxy*-1 : 1′-*dianthryl* as beautiful prismatic needles, m. p. 260° (decomp.) (0·93 g.) (Found: C, 86·7; H, 4·7. $C_{28}H_{18}O_2$ requires C, 87·0; H, 4·6%).

Deamination of Active 2 : 2′-*Diamino*-1 : 1′-*dianthryl*.—The procedure was as above, except that the quantity of water was reduced considerably. The following quantities were used: *dextro*-diamine (2·5 g.), concentrated sulphuric acid (1 c.c.), sodium nitrite (0·8 g.), distilled water (5 drops). The separated crystalline material was completely soluble in boiling toluene (rapid crystallisation), yielding 0·83 g. of *dextro*-1 : 1′-dianthryl, m. p. 283—285°, $[\alpha]_{5461}^{20°}$ +221° (*c*, 1·0 in chloroform).

Similar treatment of the *lævo*-diamine (1·5 g.) yielded 0·52 g. of *lævo*-1 : 1′-dianthryl as small colourless plates, m. p. 280—283°, $[\alpha]_{5461}^{19°}$ −212° (*c*, 0·98 in chloroform). Slight racemisation had occurred in the crystallisation.

Racemisation Experiments.—A solution of *dextro*-1 : 1′-dianthryl ($[\alpha]_{5461}^{20°}$ +260°; *c*, 0·10) in xylene was boiled under reflux for 2 hours. The resulting solution was completely inactive; the recovered material had m. p. 319—320° and gave no depression with inactive 1 : 1′-dianthryl.

A solution of *dextro*-1 : 1′-dianthryl in chloroform was heated under reflux (temperature of the solution, 63°). The solution was rapidly chilled periodically and the rotation observed. $[\alpha]_{5461}^{19—21°}$ were: initial, 4·40°; ½ hour, 3·52°; 1 hour, 2·48°; 3 hours, 1·36°; 5 hours, 0·42°.

Anthrapinacol.—Anthrone (50 g.) was reduced by the procedure of Newman (*J. Amer. Chem. Soc.*, 1940, **62**, 1683), and the product worked up by steam-distillation of the solvent. The residual white crystalline powder (48 g., 98%) was almost pure anthrapinacol. Crystallisation from benzene gives fine white needles, m. p. 185°.

9 : 9′-*Dianthryl*.—The above pinacol can be converted into 9 : 9′-dianthryl in 73% yield by the method of Bergmann and Schuchardt (*loc. cit.*). On dissolution in warm thionyl chloride, the pinacol yields α-anthrapinacolin, m. p. 219° (Barnett and Matthews, *J.*, 1923, **123**, 380); with boiling acetic anhydride it gives anthracene (cf. the corresponding reaction with 10 : 10′-dihydroxy-9 : 10 : 9′ : 10′-tetrahydro-9 : 9′-dianthryl).

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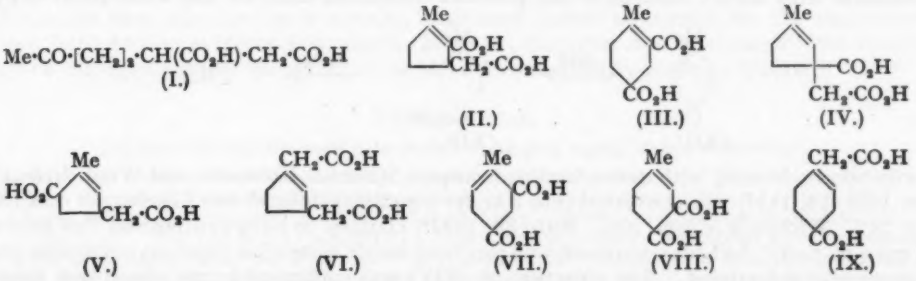
338. *Lanceol, a Sesquiterpene Alcohol from the Oil of* Santalum lanceolatum. *Part II. Some Observations on the Degradation Product.*

By L. N. OWEN.

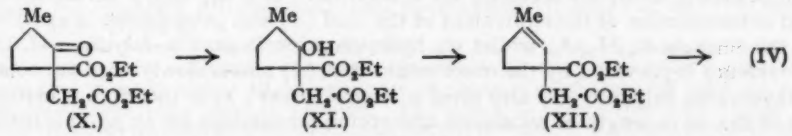
Syntheses of 3-*methyl*cyclo*pent*-2-*ene*-1-*carboxylic*-1-*acetic acid* (IV) and of 4-*methyl*cyclo*hex*-3-*ene*-1 : 3-*dicarboxylic acid* (III) are described. A compound synthesised by Bradfield, Francis, Penfold, and Simonsen (*J.*, 1936, 1619), originally thought to be the acid (III), is probably the βγ-unsaturated isomer. The acid obtained by ozonolysis of lanceol is shown to be a keto-acid, $C_{11}H_{14}O_5$ (compare Bradfield *et al.*, *loc. cit.*), six possible structures for which are discussed, the most probable being (XV).

THE sesquiterpene alcohol, lanceol, $C_{15}H_{24}O$, was shown by Bradfield, Francis, Penfold, and Simonsen (*J.*, 1936, 1619) to give on oxidation an unsaturated acid (*A*), m. p. 174°, to which the formula $C_9H_{12}O_4$ was allocated, and it was further observed that ozonolysis of this acid yielded hexan-5-one-1 : 2-dicarboxylic acid (I). On this basis there are eight possible structures, (II)—

(IX), for the C_9-acid, and these authors described the synthesis of two of these, viz. (II) and (III), neither of which was identical with (*A*); they considered that, of the remaining possible *cyclo*pentene structures, (IV) was the most likely. At the suggestion of Sir John Simonsen, a study of the synthesis of this acid was begun several years ago but had to be temporarily abandoned; more recently, it has been resumed, and the results have led to a re-examination of the whole problem.



Ethyl 2-carbethoxy-5-methyl*cyclo*pentan-1-one-2-acetate (X) (Bradfield *et al.*, *loc. cit.*) is not affected by treatment with aluminium amalgam in ether (private communication from Sir John Simonsen) and, although it appeared to be reduced under the Ponndorf conditions with aluminium *iso*propoxide, the product was not homogeneous, probably owing to the occurrence of simultaneous alcoholysis. Catalytic hydrogenation in the presence of Raney nickel, however, gave *ethyl 2-carbethoxy-5-methyl*cyclo*pentan-1-ol-2-acetate* (XI) in good yield. Considerable difficulty was experienced in the subsequent dehydration, and after unsuccessful trials with thionyl chloride and pyridine, with which only sulphur-containing products were obtained, recourse was had to the use of phosphoric oxide in a benzene solution of the hydroxy-ester; this



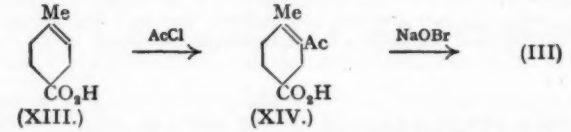
effected partial dehydration and gave a mixture of the required unsaturated ester (XII) with some unchanged (XI). Attempts to carry the dehydration to completion by prolonging the reaction led only to considerable decomposition, and the mixed esters, which could not be satisfactorily separated by distillation, were therefore hydrolysed, and the resulting acids fractionated under high vacuum. A small quantity of *3-methyl*cyclo*pent-2-ene-1-carboxylic-1-acetic acid* (IV), m. p. 158—159°, was thus obtained, the main product being an oily lactone (the formation of isomeric lactonic acids is an obvious side-reaction). The overall yield of (IV) was extremely poor, but its structure was confirmed by ozonolysis to (I); it was clearly not identical with the acid (*A*).

Before proceeding to a consideration of the syntheses of any of the remaining possibilities, the acid (*A*) was subjected to a routine spectrographic examination, and, quite unexpectedly, it showed an absorption band at $\lambda_{max.}$ 2390 A. ($E^{1\%}_{1\,cm.}$ 560). Of the eight structures, only (II) and (III) would be expected to show selective high-intensity absorption above 2000 A.* and, as mentioned above, syntheses of both of these acids had been described by Bradfield *et al.* When, however, the original specimens were examined, it was found that although the acid (II), m. p. 200—202°, showed the expected absorption ($\lambda_{max.}$ 2290 A.; ε 13,800 in alcohol), the acid of m. p. 193—195°, to which the structure (III) had been assigned, showed no selective absorption in this region and consequently could not possess that formula; it nevertheless had given (I) on ozonolysis and must therefore be formulated as the $\beta\gamma$-unsaturated isomer (VII). Support for this conclusion is afforded by the observation that on heating with concentrated aqueous alkali it was partly isomerised into the $\alpha\beta$-form (III), since the product then showed $\lambda_{max.}$ 2280 A. (ε 5000). Bradfield *et al.* (*loc. cit.*, p. 1622) mentioned that the yield of the *cyclo*hexene acid was extremely low, most of the product appearing in the aromatised form, and it is clear that under the vigorous conditions necessary to effect hydrolysis of their cyanide the position of the double

* The wave-length of maximum absorption is somewhat higher than that normally found for $\alpha\beta$-unsaturated acids; an explanation of this anomaly is given later.

bond cannot be regarded as fixed; by an unusual coincidence the αβ- and βγ-unsaturated isomers give the same products on ozonisation.

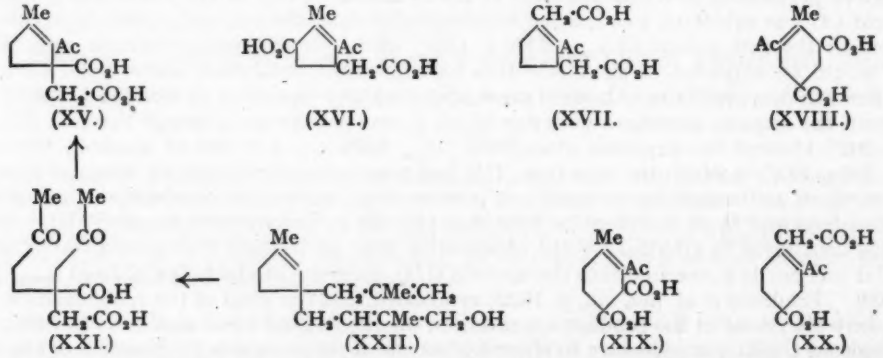
Unfortunately, the amount of the original *cyclo*hexene acid, m. p. 193—195°, was insufficient to enable the αβ-unsaturated isomer (III) to be isolated from the alkaline-equilibration mixture, and it was necessary to devise a new synthesis. This was readily achieved by the following route: 4-methyl*cyclo*hex-3-ene-1-carboxylic acid (XIII) (Lehmann and Paasche, *Ber.*, 1935, **68**, 1068), on reaction with acetyl chloride in the presence of stannic chloride and subsequent dehydro-



chlorination by heating with diethylaniline (compare Ruzicka, Koolhaas, and Wind, *Helv. Chim. Acta*, 1931, **14**, 1151) was converted into 3-*acetyl*-4-*methyl*cyclo*hex*-3-*ene*-1-*carboxylic acid* (XIV), m. p. 78°. Wichterle (*Chem. Abs.*, 1945, **39**, 1841[9]) claimed to have synthesised this keto-acid, and gave m. p. 97°, but his compound was prepared under somewhat vigorous conditions and its structure was not proved. The structure of (XIV) was confirmed by its absorption spectrum and by ozonolysis to (I). It was smoothly oxidised with sodium hypobromite to give 4-*methyl*-cyclo*hex*-3-*ene*-1 : 3-*dicarboxylic acid* (III), m. p. 230°, which showed the expected absorption maximum at 2240 A., the structure being also confirmed by ozonolysis.

It is thus established that the acid (*A*) is not identical with either of the two possible αβ-unsaturated acids (II) and (III), and it is necessary to consider more closely the evidence on which the C_9-formula of Bradfield *et al.* is based. The analytical figures given by these authors (Found: C, 58·9; H, 6·3) are in fair agreement with a formula $C_{11}H_{14}O_5$ (requires C, 58·4; H, 6·2%), although the analysis quoted for the *p*-phenylphenacyl ester supports the original C_9-formulation. Only a trace of the original ester was available, but a new preparation, which had the same m. p. (106°), analysed correctly for a derivative of a C_{11} acid; furthermore, a fresh analysis and determination of the equivalent of the acid (*A*) also gave figures in excellent agreement with the formula $C_{11}H_{14}O_5$, whilst on hydrogenation it gave a *dihydro-acid*, $C_{11}H_{16}O_5$. Conclusive evidence is provided by the observation that (*A*) reacts slowly with aqueous 2 : 4-dinitrophenylhydrazine sulphate and also gives a *semicarbazone*; it is therefore a keto-acid, and the position of the wave-length of maximum absorption (anomalous for an αβ-unsaturated acid) indicates that the double bond is conjugated with the carbonyl group.

There are six possible structures (XV)—(XX) [derived by introducing an acetyl group at one end of the double bond in each of the C_9-acids (IV)—(IX)], which agree with these facts and also allow of the formation of (I) on ozonolysis. Bradfield *et al.* pointed out that the acid (*A*) might be a secondary product of the ozonisation of lanceol. On the new formulation this would appear to be even more probable, since the acids (XV)—(XX) could be derived by an intra-molecular aldol condensation from the appropriate diketones, *e.g.*, (XXI). On this basis, formulæ (XVI), (XVII), (XVIII), and (XX), the precursors of which would be β-keto-acids, are perhaps less likely that the other two. The evidence from the absorption spectra supports (XV) rather than (XIX), since the latter, containing the 2-acetyl-1-methyl*cyclo*hexene system, would be expected



to show a much lower intensity of maximum absorption than that actually observed (ε 13,000); furthermore, the semicarbazone shows maximum absorption at 2680 A., thus differing

considerably from the semicarbazone of 2-acetyl-1-methyl*cyclo*hexene ($\lambda_{max.}$ 2350 and 2450 A.) (compare Braude, Jones, Sondheimer, and Toogood, this vol., p. 607; Braude, Jones, Koch, Richardson, Sondheimer, and Toogood, *ibid.*, in the press). Formula (XV) is also in agreement with the stability of the acid towards sulphuric acid (Bradfield *et al.*, *loc. cit.*) and accounts for the low reactivity towards 2 : 4-dinitrophenylhydrazine, since the carbonyl group is sterically hindered. It is of interest that Bradfield *et al.* postulated a keto-acid of structure (XV) as a possible intermediate in their scheme of degradation to the supposed C_9 acid, and the structure (XXII) which they advanced as a working basis may indeed be correct for the sesquiterpene alcohol; until further evidence is available, however, the other five formulæ for the keto-acid cannot be disregarded, and the structure of lanceol consequently remains uncertain.

Experimental.

(Absorption spectra were determined in alcohol unless stated otherwise).

*Reduction of Ethyl 2-Carbethoxy-5-methyl*cyclo*pentan-1-one-2-acetate.*—(*a*) The keto-ester (2·8 g.) (Bradfield *et al.*, *loc. cit.*) and aluminium *iso*propoxide (3 g.) in *iso*propanol (30 c.c.) were heated under almost total reflux, the temperature of the oil-bath being adjusted so that the distillation rate was *ca.* 2 c.c. per hour. After 30 hours (during which time more *iso*propanol was added when necessary) no more acetone appeared to be distilling, and the remaining solvent was then removed under reduced pressure. The residue was dissolved in dilute hydrochloric acid, the oil removed in ether, and the aqueous portion extracted twice with ether. The combined ethereal solutions were evaporated to an oil, which on distillation gave a main fraction (3·3 g.), b. p. 195°/17 mm., $n_D^{22°}$ 1·4340 (Found : C, 62·2; H, 8·9%), probably consisting mainly of the di*iso*propyl ester of the hydroxy-acid ($C_{18}H_{32}O_5$ requires C, 62·9; H, 9·2%); the high yield also indicates the occurrence of alkoxy-interchange.

(*b*) The keto-ester (5·5 g.) and Raney nickel (*ca.* 2 g.) in ethanol (100 c.c.) were stirred under hydrogen at 100°/50 atm. for 6 hours. The filtered solution was evaporated to an oil, which on distillation furnished *ethyl 2-carbethoxy-5-methyl*cyclo*pentan-1-ol-2-acetate* (XI) (5·0 g.), b. p. 166—168°/15 mm., $n_D^{20°}$ 1·4610 (Found : C, 60·5; H, 8·4. $C_{13}H_{22}O_5$ requires C, 60·4; H, 8·6%).

Dehydration of the Hydroxy-ester (XI).—The ester (4·75 g.) was gradually added to a stirred suspension of phosphoric oxide (5 g.) in benzene (30 c.c.). After being stirred at room temperature for 2 hours and heated under reflux for a further 20 minutes, the solution was filtered and evaporated to an oil. Distillation gave 2·5 g., b. p. 162°/16 mm., $n_D^{18°}$ 1·4580, which was unsaturated towards alkaline permanganate but evidently still contained some hydroxy-ester (Found : C, 63·7; H, 8·2. Calc. for $C_{13}H_{20}O_4$: C, 65·0; H, 8·4%).

*3-Methyl*cyclo*pent-2-ene-1-carboxylic-1-acetic Acid* (IV).—The above unsaturated ester (2·5 g.) was heated under reflux with methanolic potassium hydroxide (50 c.c.; 0·7 N.) for 7 hours. The cooled solution was then treated with a slight deficiency of aqueous sulphuric acid (49·5 c.c.; 0·7 N.) and evaporated to dryness under reduced pressure. Extraction of the residue with boiling dry acetone furnished an oil, which distilled at 100—120° (bath temp.)/10^{-5} mm. (1·8 g.), and partly crystallised after a year. The solid *acid* was drained on porous tile and recrystallised from water; it formed needles (0·1 g.), m. p. 158—159° (Found : C, 59·0; H, 6·8. $C_9H_{12}O_4$ requires C, 58·7; H, 6·6%); it rapidly reduced alkaline permanganate, and on ozonolysis in aqueous sodium carbonate solution gave hexan-5-one-1 : 2-dicarboxylic acid, m. p. 118°. The liquid portion appeared to consist mainly of an isomeric lactonic acid [Found : equiv. (by direct titration with aqueous alkali), 149, (by heating with excess alkali), 93. $C_9H_{12}O_4$ requires equiv. (as lactonic acid), 184, (as dibasic acid), 92].

*Action of Alkali on 4-Methyl*cyclo*hex-4-ene-1 : 3-dicarboxylic Acid* (VII).—The acid (30 mg.), m. p. 193—195°, in 25% aqueous potassium hydroxide (0·3 c.c.) was heated in a sealed tube for 10 hours at 100°. The cooled solution was acidified with excess of hydrochloric acid and evaporated to dryness. Extraction of the residue with dry acetone gave a solid acid (25 mg.), m. p. 160—180°, which crystallised from water in nodules, m. p. 175—180°. Light absorption : $\lambda_{max.}$ 2280 A.; ε, 5000.

*3-Acetyl-4-methyl*cyclo*hex-3-ene-1-carboxylic Acid* (XIV).—Finely powdered 4-methyl*cyclo*hex-3-ene-1-carboxylic acid, m. p. 98—99° (16·5 g.) (Lehmann and Paasche, *loc. cit.*), was added in small portions to a stirred solution of stannic chloride (30 g.) and acetyl chloride (10 g.) in carbon disulphide (100 c.c.) at −15°, a viscous lower layer gradually separating. Stirring was continued while the mixture was allowed to warm to room temperature, and next morning the complex was decomposed by the addition of crushed ice. Chloroform (100 c.c.) was added, the lower organic layer separated, and the aqueous portion extracted twice with more chloroform. The combined chloroform extracts were washed with water, dried ($CaCl_2$), and evaporated to a brown oil (17 g.), which was heated with diethylaniline (20 g.) at 180° for 3 hours; the mixture was then cooled, dissolved in excess of dilute hydrochloric acid, and extracted four times with ether. The ethereal extracts were washed with dilute hydrochloric acid and then with water, dried ($CaCl_2$), and evaporated to an oil, distillation of which gave (i) 6·6 g. of unchanged starting material, b. p. 110—112°/0·5 mm., which solidified, and (ii) 2·5 g., b. p. 140—145°/0·1 mm., which slowly crystallised when kept and on recrystallisation from benzene–light petroleum (b. p. 40—60°) formed needles of *3-acetyl-4-methyl*cyclo*hex-3-ene-1-carboxylic acid*, m. p. 78°, unchanged on further recrystallisation (Found : C, 66·1; H, 8·0. $C_{10}H_{14}O_3$ requires C, 65·9; H, 7·75%). Light absorption : $\lambda_{max.}$ 2440 A.; ε, 7500. The comparatively low intensity is characteristic of a 2-substituted acetyl*cyclo*hexene (cf. Braude *et al.*, *loc. cit.*). Ozonisation of the keto-acid in aqueous sodium carbonate solution gave hexan-5-one-1 : 2-dicarboxylic acid, which crystallised from ethyl acetate in prisms, m. p. and mixed m. p. 118—119°.

*4-Methyl*cyclo*hex-3-ene-1 : 3-dicarboxylic Acid* (III).—A solution of sodium hypobromite, prepared by the addition of bromine (1·35 g.) to sodium hydroxide (1 g.) in water (8 c.c.) at −5° was added to the keto-acid (0·5 g.) in a solution of sodium hydroxide (0·15 g.) in water (3 c.c.) at −5°, the temperature

being kept below 0° during the addition. The solution was then set aside at room temperature for 2 hours, and the slight excess of hypobromite was removed by the addition of a trace of sodium hydrogen sulphite. The lower layer of bromoform was diluted with several volumes of light petroleum to facilitate its removal, and the aqueous portion was then acidified with concentrated hydrochloric acid. A white solid separated, which was collected and recrystallised from boiling water (25 c.c.) to give fine needles (0·46 g.) of 4-*methyl*cyclo*hex*-3-*ene*-1 : 3-*dicarboxylic acid*, m. p. 230° (Found : C, 58·9; H, 6·8. $C_9H_{12}O_4$ requires C, 58·7; H, 6·6%). Light absorption : $\lambda_{max.}$ 2240 A.; ε, 10,700. It was practically insoluble in cold, but soluble in about 50 parts of boiling water. On ozonolysis in sodium carbonate solution it gave hexan-5-one-1 : 2-dicarboxylic acid, m. p. and mixed m. p. 118—119°.

Structure of the Acid (A).—The acid recrystallised from water in plates, m. p. 174° (Found : C, 58·45; H, 6·4; equiv., 113·5. $C_{11}H_{14}O_5$ requires C, 58·4; H, 6·2%; equiv., 113). Light absorption : $\lambda_{max.}$ 2390 A.; ε, 13,000. The di-*p*-phenylphenacyl ester, crystallised from methanol, had m. p. 105—106° (Found : C, 76·05; H, 5·8. Calc. for $C_{39}H_{34}O_7$: C, 76·2; H, 5·6%). The *semicarbazone* was prepared by keeping the acid (50 mg.), semicarbazide hydrochloride (35 mg.), and a slight excess of sodium acetate in water (1 c.c.), at room temperature during 10 days; after acidification with concentrated hydrochloric acid (0·1 c.c.) it slowly separated as a crystalline powder which crystallised from hot water in prisms (30 mg.), m. p. 154° (Found : C, 49·4; H, 6·4; N, 14·3. $C_{12}H_{17}O_5N_3,\frac{1}{2}H_2O$ requires C, 49·3; H, 6·2; N, 14·4%). Light absorption : $\lambda_{max.}$ 2680 A.; ε, 24,000 (in dioxan).

Hydrogenation.—The acid (*A*) (0·2 g.) in 50% acetic acid (3 c.c.) was shaken with hydrogen in the presence of a 10% palladium–charcoal catalyst (50 mg.). Absorption (20 c.c.) was complete in ½ hour. The solution was evaporated, to give a solid residue of the *dihydro-acid*, which crystallised from ethyl acetate–light petroleum in prisms, m. p. 138° (Found : C, 58·0; H, 7·1. $C_{11}H_{16}O_5$ requires C, 57·9; H, 7·1%).

The author is indebted to Sir John Simonsen, F.R.S., at whose suggestion the investigation was taken up, and who has kindly made available the original specimens. Thanks are also due to Dr. E. A. Braude for the valuable evidence afforded by the absorption spectra.

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339. *The Resorcitols. Part I.*

By W. RIGBY.

A procedure has been evolved for the separation of almost all the *cis*- and *trans*-isomers present in commercial resorcitol (*cyclo*hexane-1 : 3-diol). Derivatives have been prepared, but a previously reported conversion of the *cis*- into the *trans*-isomer is not confirmed. *cyclo*Hex-2-enone and 3-benzoyloxy*cyclo*hexanone have been prepared and the structure of the former has been established by chemical and physical methods.

FROM the mixture obtained by hydrogenation of resorcinol (Sabatier and Mailhe, *Compt. rend.*, 1908, **146**, 1193; Lindemann and Baumann, *Annalen*, 1929, **477**, 78; Rothstein, *Ann. Chim.*, 1930, **14**, 461; Coops, Dienske, and Aten, *Rec. Trav. chim.*, 1938, **57**, 303; Dimroth and Resin, *Ber.*, 1942, **75**, *B*, 322), *cis*- and *trans*-resorcitols have been isolated by methods involving fractional crystallisation of derivatives, but the methods were not efficient. Attempts at direct crystallisation have hitherto failed (*e.g.*, Rothstein, *loc. cit.*) and it now appears that this was due to attempting to separate first the less soluble *trans*-compound; actually it seems that much of the more soluble, but more readily crystallised, *cis*-compound must be isolated before any of the *trans*-isomer can be obtained. By a crystallisation process using acetone as solvent, the separation of the isomers has now been achieved, 97% of the total resorcitols present in the original mixture being isolated as pure *cis*- (m. p. 85—85·5°) and *trans*-resorcitol (m. p. 118—118·5°). These have been characterised by the preparation of derivatives and it is to be noted that the rule that *cis*- have lower melting points than the corresponding *trans*-isomers is by no means rigidly adhered to in these derivatives; exceptions are the 1-*benzoate* 3-*phenylurethanes*, 1-*benzoate* 3-p-*nitrobenzoates*, 1-benzoate 3-(3′ : 5′-dinitrobenzoates) (Dimroth and Resin, *loc. cit.*), bis(hydrogen phthalates) (Part II, succeeding paper), and possibly the bisphenylurethanes and *diacetates* (*trans* not crystallised).

The partial conversion of *cis*-resorcitol into *trans*-resorcitol dibenzoate (Rothstein, *loc. cit.*) could not be confirmed.

Oxidation of resorcitol in acid aqueous solution at 35—40° with dichromate gave *cyclo*hex-2-enone. As it was not possible to characterise this ketone from data recorded in the literature (Kötz *et al.*, *Annalen*, 1907, **358**, 197; *J. pr. Chem.*, 1909, **80**, 499; von Braun, *Ber.*, 1926, **59**, 1999; Dimroth and Resin, *loc. cit.*; Dimroth and Stockstrom, *ibid.*, p. 326), its structure was further investigated. The molecular refractivity showed an exaltation of 1·26 units (cf. Kötz, 0·21 unit), and the ultra-violet absorptions of the ketone and of its semicarbazone were in

line with those of other αβ-unsaturated ketones. The semicarbazone could be made to melt anywhere between about 160° and 185° or sharp at 184—186° according to the conditions (cf. Kötz, 161°; von Braun, 198°); the 2 : 4-dinitrophenylhydrazone melted at 164·5° (cf. Dimroth *et al.*, 167·5—169°). An oxime could not be crystallised (Kötz gives its m. p. as 75—76°). Oxidation of the unsaturated ketone with cold aqueous alkaline permanganate gave an almost quantitative yield of glutaric acid, again indicating the Δ^2-structure; direct oxidation of resorcitol with warm aqueous permanganate gave succinic acid. Using a procedure analogous to that adopted by Aldersley, Burkhardt, Gillam, and Hindley for quinitol monoacetate (*J.*, 1940, 13), resorcitol monobenzoate was oxidised to 3-benzoyloxy*cyclo*hexanone (85% yield), which is unstable, especially if impure, decomposing to benzoic acid and a liquid from which *cyclo*hex-2-enone 2 : 4-dinitrophenylhydrazone can be prepared. The same substance has since been made by Dimroth and Resin (*loc. cit.*) who used a similar but apparently less effective method.

Experimental.

cis-*Resorcitol*.—Resorcinol was hydrogenated in aqueous solution at 90—100° in presence of a nickel catalyst. The product (resorcitol), which was kindly supplied by Messrs. Howards & Sons, Ilford, was a viscous, nearly colourless syrup with an odour of peppermint. After standing for several weeks, it became translucent and semi-solid. Resorcitol (20 g.) was extracted with boiling benzene (500 c.c.), and the solution kept for six months, whereafter the crystalline deposit (stout needles) was collected and recrystallised from acetone. A solution of resorcitol in two-thirds of its weight of acetone was seeded with the crystalline fraction and kept overnight at 0°. The solid was collected, washed with acetone, and recrystallised from acetone to yield *cis*-resorcitol as prisms, m. p. 85—85·5°, b. p. 137°/13 mm., 125°/5 mm. (yield of materials, m. p. 83—85·5°, 24%).

trans-*Resorcitol*.—The combined mother- and wash-liquors were concentrated to give a solution containing three-fifths of its weight of solute; the solution was seeded with *cis*-resorcitol (no *trans*-isomer being available at this time) and stirred at −10° until thick with crystals (*ca.* 3 hours) and then at 0° for 30 minutes. The solid was collected, washed with ether–acetone (1 : 1) and then sodium-dried ether, and recrystallised from acetone. *trans*-Resorcitol separated from acetone or ethyl acetate as small prisms, m. p. 118—118·5°, b. p. 135°/13 mm. (yield of materials m. p. 112—115°, 16%). Further crops of, alternately, the *cis*- and *trans*-isomers were obtained from the ether-freed mother-liquors by concentrating so that the ratio (by weight) of acetone to solute was 1 : 1·75, cooling to −5° to −10° and stirring for 1½—1¾ hours after seeding with 2 g. of the appropriate isomer. The crops thus obtained were, after washing with acetone and sodium-dried ether (1 : 1), almost pure. Prolonged stirring tended to give a mixture of isomers, melting at *ca.* 60°; in such cases it was best to redissolve the material in the mother-liquors and repeat the crystallisation. Accumulation of the impurities of commercial resorcitol in the mother-liquors decreased the tendency of the low-melting mixture to separate; on that account although these impurities could be removed (by distillation) at any stage in the series of crystallisations, it was found undesirable to reduce their concentration to below 10% of the weight of resorcitol being crystallised.

By crystallising *cis*- and *trans*-isomers alternately, more than 57% of the resorcitol present could be isolated in 10 crystallisations. In an experiment, the object of which was to determine the relative proportions of *cis*- and *trans*-isomers in the original hydrogenation product, and which was continued to nearly 60 crystallisations (including recrystallisations), 97% of the resorcitol present was obtained in the form of pure, recrystallised isomers. The ratio *cis*- : *trans*-resorcitol was thus found to lie between the limits 1·21 : 1 and 1·35 : 1, the uncertainty depending on the composition of the residual 3% of syrupy resorcitol. Total yields were: *cis*-, 49% and *trans*-isomer, 38%; uncrystallised resorcitol, 2½%; low-boiling impurities (b. p. <135°/13 mm.) other than water, 4%; high-boiling impurities (b. p. >140°/13 mm.) 5½%. This was, however, an early experiment, and the 60 crystallisations would have been reduced by about half if the technique described above had been known.

Derivatives.—cis-*Resorcitol diacetate* separated as prisms, m. p. 36·5—36·8°, b. p. 179°/90 mm., from alcohol (Found: C, 59·8; H, 7·8. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·1%). The trans-*diacetate* is a colourless oil, b. p. 178°/90 mm., 208°/245 mm. (Found: Ac, 43·0. $C_{10}H_{16}O_4$ requires 2Ac, 43·0%).

Monobenzoates. *trans*-Recorcitol dibenzoate (obtained by use of pyridine), m. p. 123—124° (literature, 121—123°, 122·5°) (0·2 mol.), in alcohol (350 c.c.) and water (200 c.c.) was treated, slowly and with stirring, with potassium hydroxide (0·2 mol.) in water (100 c.c.). The solution was kept at 75—80° until no longer alkaline to phenolphthalein. The mixture was diluted with water (300 c.c.) and cooled, and a small amount (5 g.) of dibenzoate removed by filtration. The filtrate was extracted with chloroform, the dried extract evaporated, and the product distilled to yield trans-*resorcitol monobenzoate* as a colourless viscous liquid, b. p. 146—147°/2 mm. (Found: Bz, 48·0. $C_{13}H_{16}O_3$ requires 1Bz, 47·7%). The cis-*monobenzoate*, similarly prepared from the *cis*-dibenzoate, m. p. 66—66·5° (literature 65°, 65·5°), is a colourless oil (Found: Bz, 47·5%).

cis-*Resorcitol* 1-*benzoate* 3-p-*nitrobenzoate* separated from alcohol as almost colourless prisms, m. p. 125—127·5° (Found: C, 64·5; H, 5·4; N, 3·6. $C_{20}H_{19}O_8N$ requires C, 65·3; H, 5·2; N, 3·8%). trans-*Resorcitol* 1-*benzoate* 3-p-*nitrobenzoate* forms almost colourless prisms, m. p. 97—97·5°, from alcohol (Found: equiv., 184·5. $C_{20}H_{19}O_8N$ requires equiv., 184·5).

trans-*Resorcitol* 1-*benzoate* 3-*phenylurethane* forms prisms, m. p. 112—112·5°, from alcohol (Found: C, 70·7; H, 6·1: N, 4·5. $C_{20}H_{21}O_4N$ requires C, 70·8; H, 6·2; N, 4·1%). The cis-*isomer* forms microscopic prisms, m. p. 167·5—168·5°, from pyridine–alcohol (Found: C, 72·2; H, 6·41; N, 4·45%).

cyclo*Hex-2-enone*.—*cis*-Resorcitol (58 g.) in sulphuric acid (34 g.) and water (130 c.c.) was covered with alcohol-free ether (200 c.c.) and treated slowly with a solution of sodium dichromate (49·5 g.) in water (75 c.c.), with stirring, at 35—40°. When the addition was complete, the mixture was maintained

at 60° for 5 minutes. The mixture separated into three layers. The two lower layers were separated and extracted with ether (5 × 20 c.c.). The extract was washed with water and then sodium hydrogen carbonate solution, and dried (Na_2SO_4). Removal of the ether, followed by distillation, gave a major fraction, b. p. 100—110°/90 mm., which on redistillation yielded *cyclo*hex-2-enone (11·25 g.), b. p. 105°/100 mm., 175°/760 mm., $d_4^{20°}$ 0·9931, $[R]_L$ 28·51 (calc. 27·25), $n_D^{20°}$ 1·4892. A further quantity of the unsaturated ketone was obtained from the aqueous liquids (total yield, 22 g.). *cyclo*Hex-2-enone develops a brown colour when kept at room temperature for a few days, but remains colourless when stored at 0°. It has a burning taste and a characteristic penetrating odour, and is sternutatory (Found: C, 73·8; H, 8·5. Calc. for C_6H_8O: C, 74·95; H, 8·4%). Its light absorption in alcohol has maxima at 3230 A. (ε, 30) and 2240 A. (ε, 12,200) and a minimum at 2700 A. (ε, 6). *cyclo*Hexenone semicarbazone separates as colourless prisms from alcohol; the m. p. was indefinite, about 165—170°, but the resolidified melt melted sharply at 184—186°; this derivative has light absorption in alcohol: maximum at 2640 A. (ε, 21,000). *cyclo*Hexenone 2 : 4-dinitrophenylhydrazone separated as deep-orange-red needles, m. p. 164·5°, from *n*-butanol–ethanol (Dimroth and Resin, *loc. cit.*, give m. p. 167·5—169°) (Found: C, 52·5; H, 4·6; N, 20·5. Calc. for $C_{12}H_{12}O_4N_4$: C, 52·2; H, 4·4; N, 20·3%).

3-*Benzoyloxy*cyclo*hexanone*.—A solution of *cis*- or *trans*-resorcitol monobenzoate (8·8 g.) in glacial acetic acid (15 c.c.) was treated with chromium trioxide (3·1 g.) in water (2 c.c.) and acetic acid (8 c.c.) at 0—5°. After being kept overnight, the solution was neutralised by 20% sodium hydroxide solution, the temperature being maintained below 10° throughout. The mixture was extracted with chloroform, and the extract dried (Na_2SO_4) and evaporated. The oily product crystallised on standing and was recrystallised from benzene–light petroleum, from which 3-benzoyloxy*cyclo*hexanone (7·3 g., 83%) separated as minute prisms, m. p. 61—62°. Wasteful recrystallisation from benzene gave the pure substance, m. p. 63·5° (Dimroth *et al.*, *loc. cit.*, give m. p. 61—62°) (Found: C, 71·3; H, 6·3. Calc. for $C_{13}H_{14}O_3$: C, 71·6; H, 6·5%). After a year, a specimen had decomposed into benzoic acid and a mobile liquid which contained *cyclo*hex-2-enone, characterised as its 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 164·5°.

Oxidation of cyclo*Hex-2-enone*.—Finely powdered potassium permanganate (5·77 g.) was added gradually to a mixture of *cyclo*hex-2-enone (0·96 g.), water (5 g.), and crushed ice. The mixture was neutralised, filtered, acidified with hydrochloric acid (Congo-red), and evaporated to dryness, and the residue extracted with acetone. The extract gave glutaric acid (1·32 g.), m. p. and mixed m. p. 94—96°.

Oxidation of cis-*Resorcitol*.—A solution of *cis*-resorcitol (2·32 g.) and sodium carbonate (0·05 g.) in water (20 c.c.) was treated with powdered potassium permanganate (12·7 g.) at 65—70°. When reduction was complete, the mixture was treated as described above, to yield succinic acid (1·6 g.), m. p. and mixed m. p. 185—186°.

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340. *The Resorcitols. Part II. Resolution of* trans-*Resorcitol.*

By W. RIGBY.

The higher-melting form of resorcitol has been resolved by crystallisation of alkaloidal salts of the bis(hydrogen phthalate) thus proving it to be the *trans*-isomer.

ARGUING from the melting points, solubilities, and crystalline forms, and from the mixed melting points of the two dibenzoyl derivatives, Rothstein and co-workers (*Ann. Chim.*, 1930, **14**, 587—591) tentatively assigned the *cis*-configuration to the resorcitol (*cyclo*hexane-1 : 3-diol) isomer of lower melting point, the other being a (±)-*trans*-form. Although the method of proving the structures by resolution of the *trans*-form had been considered (Lindemann and Baumann, *Annalen*, 1929, **477**, 84, 95; Palfray and Rothstein, *Compt. rend.*, 1929, **189**, 188; Rothstein, *Ann. Chim.*, 1930, **14**, 531), no resolution has hitherto been accomplished. Lindemann and Baumann obtained a 55% yield of impure (−)-menthylurethane of the lower-melting isomer, and thence inferred that the substance was the *cis*-isomer. This was inconclusive, and further we now find that the *monobrucine* salt of (±)-*trans*-resorcitol bis(hydrogen phthalate) is similarly unchanged in rotation when recrystallised.

That the isomer of higher melting point has the *trans*-configuration has now been proved by resolution by use of alkaloidal salts of the bis(hydrogen phthalate) (cf. Pickard and Kenyon, *J.*, 1911, **99**, 58; Houssa and Kenyon, *J.*, 1930, 2261). An attempt to use the bismenthoxyacetates was abandoned as the esters did not crystallise.

When (±)-trans-*resorcitol bis(hydrogen phthalate)* was treated with sufficient brucine to give the dibrucine salt and fractionally crystallised, it gave the *monobrucine* salt of (−)-*trans*-resorcitol bis(hydrogen phthalate) (I), and a second salt (II) which appeared to be the *brucine*

salt of a mono(hydrogen phthalate). (I) yielded impure (−)-trans-*resorcitol bis(hydrogen phthalate)* and thence (−)-trans-*resorcitol*, m. p. 117·5—118°, $[\alpha]_D^{20°}$ −15·2°. Comparison with the (+)-isomer indicated that this was not quite pure. The *distrychnine* salt of *trans*-(+)-resorcitol bis(hydrogen phthalate) was prepared from the residues from the isolation of (I) and (II), and was similarly converted through the incompletely purified *bis(hydrogen phthalate)* into (+)-trans-*resorcitol*, m. p. 117·5—118·5°, $[\alpha]_D^{20°}$ +16·2°. Use of sufficient brucine to form only the monobrucine salt was unsuccessful, rotations ([α] *ca.* −10°) of the crystals deposited in successive crystallisations giving no indication of separation into diastereoisomers.

Similar treatment of cis-*resorcitol bis(hydrogen phthalate)* gave a *dibrucine* salt which could not be separated into diastereoisomers.

The bis(hydrogen phthalates) of *cis*- and (±)-*trans*-resorcitol are of interest in that the *cis*-compound has the higher melting point and lower solubility. The solubility of optically active *trans*-resorcitol in acetone at 20° is between 8 and 10%, thus resembling that of the (±)-material which has been recorded as 7·935% in acetone at 17·5° (Rothstein, *loc. cit.*). No depression in melting point was observed when about equal quantities of (+)- and (±)-*trans*-resorcitol were mixed. The specific rotations of the resorcitols are markedly lower than those of the *cyclo*hexane-1 : 2-diols (±46·5°; Wilson and Read, *J.*, 1935, 1269).

Experimental.

[α] was measured in 10% solution in B.P. chloroform unless otherwise indicated.

Resorcitol Bis(hydrogen Phthalates).—Phthalic anhydride (160 g.) and *trans*-resorcitol (58 g.) in pyridine (95 g.) were heated for 2 hours in a boiling water-bath. The resulting syrup was diluted with warm water (100 ml.) and then with a mixture of concentrated hydrochloric acid (175 ml.) and warm water (200 ml.), shaken vigorously, and then rapidly cooled by the addition of crushed ice; the slightly turbid liquid was decanted from the ester which was re-extracted similarly by shaking vigorously with a mixture of concentrated hydrochloric acid (85 ml.) and warm water (250 ml.), and then with three 250-ml. portions of warm water. The sticky product was stirred with air and dried as a froth over sulphuric acid and potassium hydroxide in an evacuated desiccator. A solution in acetone soon set to a mass of crystals which, after cooling, was filtered off and washed with a little ice-cold acetone, followed by acetone–*cyclo*hexene (1 : 1) and finally light petroleum. The white crystals had the melting point of the pure substance; yield, 166·5 g. (81%). Recrystallisation from ethyl acetate by addition of *cyclo*hexene gave the pure (±)-trans-*ester* as small, white granular crystals, m. p. 156—157°, soluble in many organic solvents, but sparingly soluble in *cyclo*hexene, and almost insoluble in light petroleum (b. p. 40—60°) and in cold water (Found: C, 64·05; H, 4·64. $C_{32}H_{30}O_8$ requires C, 64·0; H, 4·9%).

The cis-*ester*, similarly prepared, formed white, granular crystals (from ethyl acetate–*cyclo*hexene or from 70% alcohol), rather less soluble than the *trans*-compound; m. p. 186—187° [Found: C, 63·9; H, 4·74; equiv. (direct titration in aqueous alcohol), 207·5. $C_{20}H_{18}O_4(CO_2H)_2$ requires equiv., 206].

Dibrucine Salt of cis-*Resorcitol Bis(hydrogen Phthalate).*—*cis*-Resorcitol bis(hydrogen phthalate) (66 g., 1 mol.) and brucine (150 g., 2 mols.) in methyl alcohol (100 ml.), kept overnight, deposited 196 g. of the *dibrucine* salt; repeated crystallisation from ethyl alcohol, chloroform, acetone, and mixtures thereof brought about no separation into diastereoisomers. The salt formed white crystals, sparingly soluble in alcohol or acetone, more soluble in chloroform; $[\alpha]_D^{20°}$ −19·1°. Placed in a bath at 85°, the temperature rising 10° per minute, the salt turned into an opaque froth at 110—115° and melted to a clear liquid at 152—157° (Found: C, 67·8; H, 6·31; N, 4·92; brucine, 65·4. $C_{32}H_{30}O_8.2C_{23}H_{26}O_4N_2$ requires C, 68·0; H, 6·04; N, 4·66; brucine, 65·6%).

Brucine Salts from (±)-trans-*Resorcitol Bis(hydrogen Phthalate).*—Acetone (150 ml.) was added to (±)-*trans*-resorcitol bis(hydrogen phthalate) (61 g.) and brucine (anhydrous, 136 g.) dissolved in methyl alcohol (50 g.). After the mixture had been kept overnight, crystals (55 g.), $[\alpha]_D^{20°}$ −31·1°, separated. Recrystallisation, effected by dissolution in acetone and clearing the turbidity with 5—10% of methyl alcohol, gave successive crops, 51 g., $[\alpha]_D^{20°}$ −31·2°, and 39·5 g., $[\alpha]_D^{20°}$ −33·25°. The last crop dissolved in acetone (85 ml.) to give a turbid solution and, when methyl alcohol (20 ml.) was added, crystallisation at once began, to give, in a few hours a compact translucent mass adhering to the bottom of the flask; this was filtered off and washed with acetone before bulky white crystals of another salt appeared. The compact crystals (D) (11 g.) had $[\alpha]_D^{20°}$ −3·25°; the filtrate (F) was reserved. Heating (D) with acetone, in which it did not dissolve, followed by recrystallisation from a (supersaturated) solution in chloroform by addition of acetone gave the *monobrucine* salt (I) of (−)-*trans*-resorcitol bis(hydrogen phthalate) as small, white granular crystals, $[\alpha]_D^{20°}$ −1·25° (constant), retaining a small amount of chloroform when dried at 100°/100 mm. in an air stream. This salt was soluble in about 6 parts of boiling chloroform, but stable supersaturated solutions were readily made by evaporation; it was sparingly soluble in acetone or methyl alcohol and melted at 203·5—205·5° when introduced into a bath at 200° [Found: C, 64·2; H, 5·62; N, 4·1; Cl, 1·38; brucine, 47·9. $9(C_{32}H_{30}O_8.C_{23}H_{26}O_4N_2).CHCl_3$ requires C, 66·0; H, 5·67; N, 3·42; Cl, 1·44; brucine, 48·2%]. Brucine was removed from 0·1500 g. of (I) by extraction with 125 and 100 ml. of benzene in the presence of 10·00 ml. of 0·1N-sodium hydroxide, the residual solution was heated under reflux for 5 hours, and the excess of alkali titrated; this gave a phthalic acid content of 40·0% (required 41·2%); the molar ratio of alkaloid to phthalic acid was 1 : 1·98.

The filtrate (F) became full of bulky white crystals, $[\alpha]_D^{20°}$ −35·25°, which, in two interrupted crystallisations from acetone containing a little methyl alcohol, gave *ca.* 7 g. of the above-mentioned monobrucine salt and 10 g. of another *salt* (II); the latter formed small needles, soluble in warm acetone, and more soluble in methyl or ethyl alcohol and in chloroform, and had $[\alpha]_D^{20°}$ −32·75°; m. p. indefinite (begins

to sinter at *ca.* 155°) (Found: C, 65·0; H, 6·36; N, 4·45; brucine, 59·6. $C_{37}H_{42}O_9N_2$ requires C, 67·5; H, 6·43; N, 4·25; brucine, 59·9%. These data agree equally with those for $C_{23}H_{20}O_8,2C_{23}H_{26}O_4N_2,4CH_3OH$).

Strychnine Salt of (+)-trans-*Resorcitol Bis(hydrogen Phthalate)*.—The filtrates left after isolation of the two brucine salts were evaporated and brucine was removed. This gave a syrup, 25 g. of which were dried, treated in chloroform with strychnine (36 g.), and evaporated until 77 g. of chloroform remained. Crystallisation was induced by addition of acetone (80 g.), and six further crystallisations from 2—4 parts of chloroform with 1 of acetone gave 9·5 g. of the *strychnine* salt, $[\alpha]_D^{20°}$ −14·35°, of the (+)-*trans*-diester. This was sparingly soluble in alcohol or acetone and fairly soluble in boiling chloroform, and readily gave supersaturated solutions in chloroform. It sintered at *ca.* 170° and melted to a slightly brown liquid at 180—182° (Found: C, 64·6; H, 5·2; N, 4·61; Cl, 10·0; strychnine, 55·8. $C_{23}H_{20}O_8,2C_{21}H_{22}O_2N_2,CHCl_3$ requires C, 65·0; H, 5·46; N, 4·67; Cl, 8·87; strychnine, 55·1%).

Bis(hydrogen Phthalate) of (−)-trans-*Resorcitol*.—Removal of the brucine from (I) (16·5 g.) by adding a slight excess of alkali, acidifying, and extracting with chloroform gave a sticky precipitate which, after washing with water, was converted into a froth and dried in a vacuum over sulphuric acid and potassium hydroxide. The almost colourless, friable product did not crystallise and was impure [Found: equiv., (by direct titration) 228, (by hydrolysis) 115. $C_{23}H_{20}O_8$ requires equiv., 206 and 103, respectively].

Bis(hydrogen Phthalate) of (+)-trans-*Resorcitol*.—The product (10·5 g.) obtained similarly from 31·5 g. of the strychnine salt described above did not crystallise. It had $[\alpha]_D^{20°}$ +14·6°, falling to +13·2° after one day [Found: equiv., (by direct titration) 242, (on hydrolysis) 123].

(−)-trans-*Resorcitol*.—The crude diester from *ca.* 16 g. of brucine salt was softened with 2—3 ml. of alcohol and heated with 10N-sodium hydroxide in slight excess for 6 hours. The slightly alkaline solution (if neutralised, the solution soon became alkaline when kept) was evaporated on the water-bath under reduced pressure until crystallisation began to spread throughout the mass. Acetone (15 ml.) was added, and the mixture was warmed and filtered, the solid being extracted with four further 10-ml. portions of acetone; evaporation and exposure in a partly evacuated desiccator gave crystalline material (2·69 g.). This was purified by recrystallisation from 30 ml. of hot ethyl acetate–benzene (1 : 4), and treating an acetone solution with charcoal; this afforded (−)-trans-*resorcitol* as fine colourless needles 2·08 g., soluble in water, alcohol, ethyl acetate, or acetone, less soluble in benzene, and almost insoluble in light petroleum; m. p. 117·5—118°; $[\alpha]_D^{20°}$ −15·2° (*c*, 10 in methyl alcohol) (Found: C, 62·5; H, 10·1. $C_6H_{12}O_2$ requires C, 62·0; H, 10·4%).

(+)-trans-*Resorcitol*.—This was similarly prepared. Soxhlet extraction of the products of hydrolysis was less satisfactory, an oil being also produced. The (+)-*isomer* had m. p. 117·5—118·5° and $[\alpha]_D^{20°}$ +16·2° (*c*, 10 in methyl alcohol) (Found: C, 61·7; H, 10·3%).

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341. *Derivatives of* 1 : 6-*Anhydro*-β-D-*idose.*

By L. F. WIGGINS.

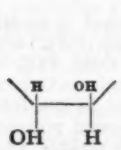
The formation of 1 : 6-anhydro-derivatives of hexoses is discussed. A new crystalline derivative of D-idose is described, namely 2 : 3 : 4 : 6-*tetra-acetyl* β-*methylidoside*.

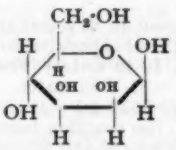
IN an earlier communication (*J.*, 1944, 522) it was shown that galactose was converted, through its 2-tosyl derivative, into 2 : 3-anhydro-β-methyltaloside. The 4 : 6-benzylidene derivative of this compound underwent ring scission with sodium methoxide to give mainly 4 : 6-benzylidene 3-methyl β-methylidoside (A), together with a little 4 : 6-benzylidene 2-methyl β-methylgalactoside (B). The formation of these two products was in accord with the accepted theory of anhydro-ring scission. In experiments designed to determine its constitution, the compound (*A*) was hydrolysed first to 3-methyl β-methylidoside, then under more drastic conditions to a product which should have been 3-methyl idose. It was observed, however, that the product possessed only slight reducing power towards Fehling's solution; moreover oxidation of the sugar with bromine did not give pure 3-methyl idonic acid or its lactone, since the derived non-crystalline amide contained much less nitrogen than the theoretical, and gave only ¼ of the expected yield of hydrazodicarbonamide on being treated with Weerman's reagent. These facts which suggested that some modification of the reducing group of 3-methyl idose impeded its oxidation with bromine have now received a ready explanation in the light of the recent work of Sorkin and Reichstein (*Helv. Chim. Acta*, 1945, **28**, 1). These authors have shown that idose tends to undergo internal anhydride formation involving the reducing group and the hydroxyl at C_4. Thus, hydrolysis of β-methylidoside led to the formation of 1 : 6-anhydro-β-idose, and thence to that of the 2 : 3 : 4-triacetate. Sorkin and Reichstein also showed that

the crude hydrolysis product of β-methylidoside reduced Fehling's solution only about one-fifth as strongly as did glucose, and that bromine oxidation of this product gave only a 20% yield of idonic acid. Thus the 3-methyl idose described in 1944 was probably a mixture of 3-methyl idose and 3-methyl 1 : 6-anhydro-β-idose. The bromine oxidation product was therefore a mixture of 3-methyl 1 : 6-anhydro-β-idose and 3-methyl idonic acid lactone, and the amide prepared therefrom also contained the 1 : 6-anhydro-compound as an impurity, thus accounting for its low nitrogen value and for the low yield of hydrazodicarbonamide obtained on treatment with Weerman's reagent.

Another example of the spontaneous formation of 1 : 6-anhydroidose has been observed. When 3-acetamido 2-acetyl 4 : 6-benzylidene β-methylidoside (Wiggins, *loc. cit.*) was hydrolysed with concentrated hydrochloric acid, the product after re-acetylation was found to be 3-acetamido 2 : 4-diacetyl 1 : 6-anhydro-β-idose, a substance already prepared by James, Smith, Stacey, and Wiggins (*J.*, 1946, 625) by the ring scission with ammonia of both 2 : 3- and 3 : 4-1 : 6-dianhydro-β-talose. Since there are now three different derivatives of idose which have been shown to undergo 1 : 6-anhydro-ring formation in aqueous solution, it is reasonable to suppose that this phenomenon is common to all derivatives of idose in which the hydroxyl groups at C_1 and C_6 are unsubstituted.

Although several hexoses, for example, D-glucose and D-mannose, form 1 : 6-anhydrides under pyrogenetic conditions, only two, namely D-idose and D-altrose, are known to undergo 1 : 6-anhydro-ring formation under ordinary conditions in solution. It is interesting to note that β-D-idose and β-D-altrose have identical arrangements of the hydrogen and hydroxyl groups about C_2 and C_3 (I), and moreover these are the only aldohexoses of the D-series which possess this arrangement. The configuration of the hydrogen and hydroxyl groups about C_1 can vary in any sugar, but clearly the sugar must be of the β-form when 1 : 6-anhydro-ring formation takes place. It would appear that neither of the particular configurations about C_2 and C_3, taken separately, is responsible for the spontaneous ring-formation, because neither β-D-mannose (II) which has the same arrangement of hydrogen and hydroxyl about C_2 as altrose and idose, nor β-D-allose (III), which has the same configuration about C_3 as altrose and idose, undergoes spontaneous 1 : 6-anhydride formation.


(I.)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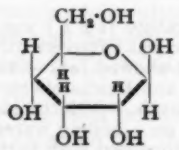
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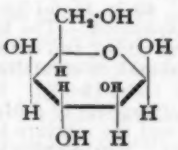
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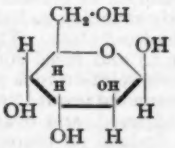
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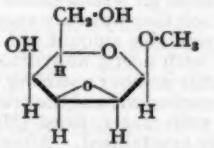
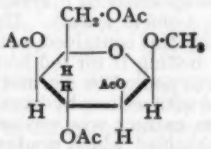

(VI.)

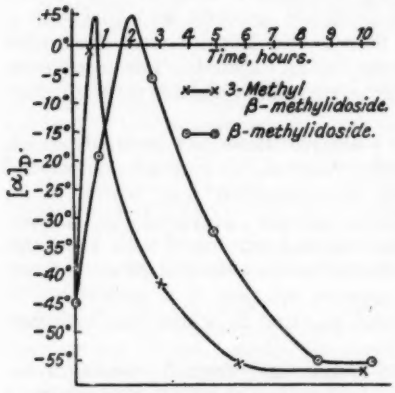

(VII.)

The position of the hydroxyl group at C_4 is evidently not critical in regard to 1 : 6-anhydro-ring formation, because the configuration at that point is different in idose (IV) and altrose (V).

It is not yet clear why this particular orientation of groups on C_2 and C_3 of an aldohexose should facilitate the formation in solution of the 1 : 6-anhydro-ring, and it can only be pointed out at this stage that this configuration does play a critical part in the phenomenon.

Sorkin and Reichstein (*loc. cit.*) obtained 1 : 6-anhydro-β-D-idose, and thence the characteristic triacetate, by hydrolysis of 4 : 6-benzylidene β-methyl-D-idoside. The same compound has now been obtained by a somewhat different route, and the observations of Sorkin and Reichstein confirmed. 2 : 3-Anhydro-β-methyltaloside (VI) (*J.*, 1944, 522) was treated with aqueous potassium hydroxide at room temperature. Ring scission took place very slowly and was complete in 792 hours. After acetylation 2 : 3 : 4 : 6-*tetra-acetyl* β-*methylidoside* (VII) was isolated in 65% yield. No derivative of β-methylgalactoside was isolated. The constitution of (VII) follows from the earlier work on the ring scission of 2 : 3-anhydro-β-methylidoside. De-acetylation of (VII) gave liquid β-methylidoside which defied all attempts at crystallisation. Sorkin and Reichstein (*loc. cit.*) also did not obtain it crystalline. The

hydrolysis of β-methylidoside by N-sulphuric acid was followed polarimetrically. It proceeded according to the graph shown in the figure, in which the hydrolysis curve of 3-methyl β-methylidoside is also shown. From the hydrolysis mixture triacetyl 1 : 6-anhydro-β-D-idose was isolated after acetylation. It had constants in close agreement with those recorded by Sorkin and Reichstein (*loc. cit.*). That the hydrolysis of both β-methylidoside and its 3-methyl derivative proceeded in a strikingly similar way is apparent from the figure. It is probable that in each case the first peak in the curves represents the removal of the glycosidic methyl group, and the subsequent fall in specific rotation indicates the formation of the 1 : 6-anhydro-ring.



Hydrolysis of β-methylidoside and its 3-methyl ether with N-sulphuric acid.

Experimental.

Hydrolysis of 2 : 3-*Anhydro*-β-*methyltaloside with Potassium Hydroxide.*—The taloside (1·005 g.), prepared according to the method described by Wiggins (*loc. cit.*), was left in the cold with 5% aqueous potassium hydroxide (25 c.c.) and the reaction followed polarimetrically: $[\alpha]_D$ −90·0° (initial value); −88·5° (3·5 hours); −85·0° (6 hours); −83·1° (23 hours); −80·0° (39 hours); −76·3° (63 hours); −67·4° (135 hours); −61·9° (189 hours); −52·7° (548 hours); −51·7° (620 hours); −50·2° (792 hours); −50·2° (840 hours). Thereafter the solution was heated at 100° for 2 hours without there being any further change in specific rotation. The solution was neutralised with N-sulphuric acid, and the mixture evaporated in the presence of a little barium carbonate, dried, and acetylated by treatment with acetic anhydride (10 c.c.) and pyridine (15 c.c.). After 48 hours at 30°, the mixture was poured into ice–water and then extracted with chloroform. The extract was washed successively with 5% sulphuric acid, dilute sodium hydrogen carbonate solution, and water. After being dried ($MgSO_4$), the solution was filtered, and the solvent removed under diminished pressure. The syrupy residue (1·7 g.) crystallised on cooling. Recrystallised from alcohol it formed feathery needles (1·3 g.; 65%) of 2 : 3 : 4 : 6-*tetra-acetyl* β-*methylidoside*, m. p. 112—113°, $[\alpha]_D^{20°}$ −64·1° (*c*, 1·56 in chloroform). A m. p. in admixture with tetra-acetyl β-methylgalactoside showed a marked depression (Found: C, 49·8; H, 6·2. $C_{15}H_{22}O_{10}$ requires C, 49·7; H, 6·1%).

β-Methylidoside.—The tetra-acetate (1·2 g.) was dissolved in dry methyl alcohol, a small piece of sodium was added, and the solution was left overnight and then evaporated to a syrup (0·5 g.) which could not be induced to crystallise. It showed $[\alpha]_D$ −40·8° (*c*, 10 in water) (Found: OMe, 15·5. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). Sorkin and Reichstein give $[\alpha]_D$ −81·1° in water.

Acid Hydrolysis of β-*Methylidoside.*—β-Methylidoside (0·3534 g.) was dissolved in N-sulphuric acid (10 c.c.), and the mixture heated on a boiling water-bath, the reaction being followed polarimetrically (see figure). The solution was neutralised with barium carbonate and filtered, the residue washed with hot water, and the combined filtrates evaporated to dryness. The residue was dissolved in a little water, filtered, and evaporated to a syrup (0·25 g.) which defied all attempts at crystallisation. It showed $[\alpha]_D$ −66·3° (*c*, 4·468 in water). The solution was only very slightly reducing to Fehling's solution, and in all probability this contained a considerable amount of 1 : 6-anhydro-β-idose. The syrup (0·2 g.) was acetylated by boiling it for 0·5 hour with acetic anhydride (10 c.c.) and fused sodium acetate (1 g.). The mixture was poured on ice, and after an hour's stirring, complete solution took place. The solution was neutralised with sodium hydrogen carbonate, and the product extracted several times with chloroform. The chloroform extract was washed with water, dried ($MgSO_4$), filtered, and evaporated. A syrupy product was obtained which gradually crystallised. After being drained on porous tile, the solid was recrystallised several times from water. The product, which crystallised in small platelets, had m. p. 85·5—86·5° and showed $[\alpha]_D$ −73·1°. These figures are in close agreement with those (m. p. 86—87°, $[\alpha]_D$ −73·6° in chloroform) given by Sorkin and Reichstein (*loc. cit.*) for triacetyl 1 : 6-anhydro-β-idose.

Hydrolysis of 3-*Acetamido* 2-*Acetyl* 4 : 6-*Benzylidene* β-*Methylidoside.*—The compound (0·183 g.) was heated at 100° with 10% hydrochloric acid (10 c.c.) for 3½ hours. The solution showed $[\alpha]_D$ −35·0° at this stage. It was then evaporated to dryness without neutralisation, and the syrup obtained was immediately acetylated by treatment with acetic anhydride (4 c.c.) and pyridine (5 c.c.) for 3 days at room temperature. The product was poured into water, the solution extracted with chloroform, and the extract washed successively with dilute sulphuric acid, sodium hydrogen carbonate solution, and water, dried ($MgSO_4$), filtered, and evaporated. A semicrystalline mass was obtained which, recrystallised from alcohol, formed stout needles (0·15 g.), m. p. 245°, $[\alpha]_D^{18°}$ −73·6° (*c*, 1·467 in chloroform). It was 3-acetamido 2 : 4-diacetyl 1 : 6-anhydro-β-idose, identical in m. p., mixed m. p., and specific rotation with that obtained by the ring scission of either 2 : 3- or 3 : 4-1 : 6-dianhydro-β-talose with ammonia (see James, Smith, Stacey, and Wiggins, *J.*, 1946, 625).

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342. *Studies on Hydrogen Cyanide. Part XVII. The Alleged Resorcylaldimine Hydrochloride.*

By Leonard E. Hinkel and Roy P. Hullin.

The reaction between resorcinol and hydrogen cyanide in presence of hydrogen chloride has been investigated and it has been shown that the alleged aldimine hydrochloride is probably a derivative of the sesquichloride of hydrogen cyanide containing two molecules of resorcinol, one of which is attached through the nucleus whilst the other is attached as an *O*-ether.

A dialdimine hydrochloride has been prepared which differs from the above compound in having both molecules of resorcinol attached through the nucleus.

The view is expressed that, in order to account for their salt-forming properties, formylimino ethers may also be derivatives of the sesquichloride of hydrogen cyanide.

Whereas Gattermann and his co-workers (*Ber.*, 1898, **31**, 1766; 1899, **32**, 278; *Annalen*, 1909, **357**, 313) considered the primary product in the hydrogen cyanide synthesis of aromatic aldehydes to be iminoformyl chloride NH:CHCl, Hinkel, Ayling, and Morgan (*J.*, 1932, 2793) regarded it as chloromethyleneformamidine NH:CH·N:CHCl and suggested that the resorcylaldimine hydrochloride of Gattermann was resorcylmethyleneformamidine hydrochloride $(OH)_2C_6H_3\cdot CH{:}N\cdot CH{:}NH,HCl$. Subsequently, however, Hinkel, Ayling, and Beynon (*J.*, 1936, 184) obtained the latter compound, as a bright yellow substance, by interaction of chloromethyleneformamidine with resorcinol.

Since the resorcylaldimine hydrochloride of Gattermann is almost white, it cannot have the structure suggested by Hinkel, Ayling, and Morgan (*loc. cit.*).

Although the yields of resorcylaldehyde obtained under Gattermann's conditions are invariably about 50% calculated on the resorcinol, yet the supernatant ether contains only a very small amount of dissolved solid. Almost the whole of the resorcinol must therefore have been used in the reaction, yielding a compound from which only a part of the original resorcinol is recoverable as resorcylaldehyde on hydrolysis.

It has now been found that this compound always yields on hydrolysis a mixture of resorcylaldehyde and resorcinol together with formic acid and ethyl ether.

It is probable therefore that part of the resorcinol is united in the molecule as a phenolic ether. Houben (*Ber.*, 1926, **59**, 2878) has shown that phenols can react with hydrogen cyanide and hydrogen chloride to yield iminomethyl ethers NH:CH·OR which are readily hydrolysed to regenerate the phenols.

The product settles out in the first instance as a thick liquid which becomes semi-solid overnight. If the supernatant ethereal solution be decanted and the thick liquid or semi-solid kept in a desiccator, the syrup becomes hard and brittle. It is significant that the product obtained in several repetitions under Gattermann's conditions, whether liquid, semi-solid, or hard, always gave the same analytical results for nitrogen and chlorine. The yield of resorcylaldehyde does vary slightly, depending on the initial temperature and on the rate at which the hydrogen chloride is passed into the reaction mixture. Thus it is possible (see Experimental) under suitable conditions, particularly at low temperature, to obtain a product in which all the combined resorcinol is recoverable as resorcylaldehyde. It is therefore highly probable that Gattermann's product is not the true aldimine hydrochloride (IV) but a derivative of the sesquichloride of hydrogen cyanide $CHCl_2\cdot NH\cdot CHCl\cdot NH_2$ having the structure (I) in combination with one molecule of ethyl ether. The compound may have associated with it varying quantities of the true aldimine hydrochloride (IV) also in combination with one molecule of ethyl ether, so that, whilst the yield of resorcylaldehyde may vary, the analytical percentages of nitrogen and chlorine remain unchanged. Furthermore, the product when kept *in vacuo* slowly loses the combined ethyl ether yielding a solid which, from its nitrogen and chlorine contents, corresponds to (I) or (IV).

The suggested structure accounts for the formation of resorcylaldehyde, resorcinol, and formic acid on hydrolysis. Its formation can be explained through the intermediates iminoformyl chloride (CHCl:NH) and formamidodichloride [dichloromethylamine], $CHCl_2\cdot NH_2$; thus the iminoformyl chloride, by reacting with resorcinol, could form an iminomethyl ether (II), whilst formamidodichloride can given rise to *ω-chloro-ω-aminomethylresorcinol* (III). These two products by condensation in the presence of free hydrogen chloride could give rise to the sesquichloride derivative (I).

It has been shown (Hinkel and Watkins, *J.*, 1944, 647) that, owing to the readiness with which formamido dichloride dissociates, on dilution, into iminoformyl chloride and its

components, the yield of aldehyde under Gattermann's conditions decreases with dilution of the reactants. It is to be expected that under these conditions imino-ether formation should occur.

$$\underset{\text{(I.)}}{(OH)_2C_6H_3\cdot CHCl\cdot NH\cdot CH(O\cdot C_6H_4\cdot OH)\cdot NH_2,HCl} \longleftarrow HCl + \underset{\text{(II.)}}{OH\cdot C_6H_4\cdot O\cdot CH{:}NH} + \underset{\text{(III.)}}{(OH)_2C_6H_3\cdot CHCl\cdot NH_2}$$

$$\underset{\text{(IV.)}}{(OH)_2C_6H_2\cdot CHCl\cdot NH\cdot CH\cdot C_6H_3(OH)_2\cdot NH_2,HCl}$$

In Gattermann's experiments, since hydrogen chloride is being gradually added to the solution of hydrogen cyanide, there must always be some iminoformyl chloride present during the initial stages, and, since apparently it is this compound which gives rise to imino-ethers, it is hardly to be expected that the final product should be the true aldimine hydrochloride.

If this view be correct, then by altering the conditions so that the reaction mixture contains only formamidodichloride, it should be possible to inhibit the formation of imino-ethers. This expectation has now been realised.

When hydrogen cyanide is added quickly at a low temperature to a concentrated solution of resorcinol in ether containing an excess of hydrogen chloride, a solid is obtained which corresponds by analysis to the *aldimine hydrochloride* (IV), which in this case is devoid of any combined solvent ether. This compound can be more readily prepared by substituting ethyl acetate for ether as a solvent.

Although hydrogen chloride is much less soluble in ethyl acetate than in ether, yet it seems that the dissociation of formamidodichloride does not occur so readily; thus, using Gattermann's conditions and quantities, but ethyl acetate, it is possible to obtain even at room temperature the anhydrous hydrochloride free from any phenolic ether.

Since the supposed resorcylaldimine hydrochloride is only slowly hydrolysed in the cold by water, it does not seem that it owes its basic properties to the C:NH group especially as in such compounds as NH:CH·NC, NH:CH·N:CHCl, NH:CH·NR_2, and NH:C(CN)·CH(CN)·NH_2 the NH:C group does not appear to exhibit any basic properties. It would therefore seem more probable that the supposed aldimine is really a dialdimine (IV).

This view raises doubts about the generally accepted structure of the hydrochlorides of formimino ethers, RO·CH:NH,HCl. It seems more probable from their mode of preparation that these compounds also are derivatives of the sesquichloride of hydrogen cyanide or di-imino ethers, RO·CHCl·NH·CH(OR)·NH_2,HCl.

Experimental.

Reactions in Ethyl Ether.—(a) *Addition of hydrogen chloride to hydrogen cyanide and resorcinol at room temperature.* The product obtained by slowly saturating a solution of resorcinol (8 g.; 1 mol.) and hydrogen cyanide (3 c.c.; 1 mol). in pure dry ether (30 c.c.) with hydrogen chloride according to the directions of Gattermann and Kobner (*Ber.*, 1899, **32**, 278) was set aside overnight and the supernatant ether was then decanted. The solvated *product* was analysed, nitrogen being determined by distillation with alkali and chlorine by precipitation with silver nitrate. The aldehyde was determined by dissolving the compound in the least amount of water and heating for ½ hour on a steam-bath to remove the combined ether and to effect hydrolysis. After the mixture had cooled, resorcylaldehyde separated as white crystals, which, after washing with a small quantity of cold water, were dried and weighed; after crystallisation from hot water it melted at 136° [Found: N, 6·5; Cl, 16·2; aldehyde, 31·9. $C_{14}H_{15}O_4N_2Cl,HCl,(C_2H_5)_2O$ requires N, 6·6; Cl, 16·8; aldehyde, 32·7%]. When the product is left in a vacuum desiccator for some weeks, it loses the combined ether yielding a hard yellowish solid [Found: N, 8·1; Cl, 20·4; aldehyde, 39·4. $C_{14}H_{15}O_4N_2Cl,HCl$ (I) requires N, 8·0; Cl, 20·46; aldehyde, 39·8%]. The filtrate from the aldehyde was thoroughly extracted with ether. After removal of ether, the dark-coloured residue yielded, by careful sublimation, crystals of resorcinol which gave the characteristic nitroso-test with sodium nitrite in acetic acid, followed by alkali. The aqueous solution after extraction with ether yielded formic acid on distillation with dilute sulphuric acid.

A repetition of the above experiment, but at a low temperature (ice–salt), yielded a cream-coloured *product* as soft crystals [Found: N, 6·6; Cl, 16·8; aldehyde, 62·4. $C_7H_8O_2NCl,0{\cdot}5(C_2H_5)_2O$ requires N, 6·6; Cl, 16·8; aldehyde, 65·5%]. These crystals, after being kept in a vacuum desiccator for many days, lose the combined ether and change into a yellow powder [Found: N, 8·1; Cl, 19·9; aldehyde, 74·9. $C_{14}H_{15}O_4N_2Cl,HCl$ (IV) requires N, 8·0; Cl, 20·46; aldehyde, 79·5%].

In an experiment at 0° the yields of the aldehyde in the product, before and after keeping in a vacuum desiccator, were 52·8 and 64·4%, respectively. The higher values for the aldehyde in the last two experiments indicate the presence of much aldimine with the first product.

(b) *Addition of hydrogen cyanide to excess of hydrogen chloride and resorcinol.* Hydrogen chloride was passed into anhydrous ether (30 c.c.), cooled to −40° (alcohol and CO_2), until the gain was 11·5 g. (1·16 mols.); resorcinol (29·8 g., 1 mol.), followed by hydrogen cyanide (9·9 c.c., 1 mol.), were then added, and the whole thoroughly mixed. The firmly stoppered * vessel with its contents was kept at

* If the temperature is allowed to rise, the reaction may proceed with explosive violence.

−40° for a day, whereupon a mass of cream-coloured crystals separated. The temperature of the mixture was allowed to rise to −25°, and the supernatant ether decanted; the crystal mass was disintegrated and rapidly washed with several portions of well-cooled anhydrous ether. The crystals (*ω-chloro-ω-aminomethylresorcinol*) were rapidly dried on a porous saucer and finally for a few minutes in a vacuum desiccator (Found: N, 7·9; Cl, 19·9; aldehyde, 78·8. $C_7H_8O_2NCl$ requires, N, 8·0; Cl, 20·46; aldehyde, 79·5%).

Experiments in Ethyl Acetate.—Since hydrogen chloride is not very soluble in ethyl acetate, the following experiments were carried out according to Gattermann's directions, by passing a stream of hydrogen chloride into an ethyl acetate solution of resorcinol and hydrogen cyanide, but varying the concentrations and temperatures.

(a) *Low temperature.* (i) Hydrogen chloride was passed, to saturation, into a solution of resorcinol (8 g., 1 mol.) and hydrogen cyanide (3 c.c., 1 mol.) in pure ethyl acetate (25 c.c.) cooled to −17°, the gain in weight being 10 g. (3 mols.). The solution remained clear; after five hours at −17° a mass of fine, almost white, solvated crystals separated. These were removed, washed with ethyl acetate, and dried *in vacuo* for two days; they then had m. p. 95° (complete at 120°). When they were dissolved in warm water ethyl acetate was evolved (Found: N, 5·4; Cl, 12·8; aldehyde, 52·2. $C_7H_8O_2NCl,C_4H_8O_2$ requires N, 5·3; Cl, 13·6; aldehyde, 52·8%).

(ii) Repetition of the above experiment, using 30 c.c. instead of 25 c.c. of ethyl acetate, yielded a *solvate* as white needles, m. p. 140° (Found: N, 6·4; Cl, 16·0; aldehyde, 63·0. $C_7H_8O_2NCl,0{\cdot}5C_4H_8O_2$ requires N, 6·4; Cl, 16·3; aldehyde, 63·4%).

(b) *Room temperature.* (i) Hydrogen chloride was passed slowly into a solution of resorcinol (8 g., 1 mol.) and hydrogen cyanide (3 c.c., 1 mol.) in pure ethyl acetate (30 c.c.) at room temperature. The absorption of hydrogen chloride was very slow; after 1 hour a light pink precipitate was formed, the passage of the gas being continued until precipitation seemed complete. The precipitate was filtered off, washed with ethyl acetate, and dried on a porous plate; a pink powder was obtained which did not melt on heating (Found: N, 8·1; Cl, 20·5; aldehyde, 78·8. Calc. for $C_7H_8O_2NCl$: N, 8·07; Cl, 20·46; aldehyde, 79·5%).

(ii) With more concentrated solutions, *e.g.*, 25 c.c. of ethyl acetate, the above reaction generates considerable heat causing secondary reactions, and the product is a deep red powder.

UNIVERSITY COLLEGE, SWANSEA. [*Received, November 24th*, 1948.]

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343. *The Suggested Identification of* cis-*Azobenzene with an Equimolecular Mixture of Hydrazobenzene and Azoxybenzene.*

By R. J. W. LE FÈVRE and P. SOUTER.

The mixture named in the title has been examined at 25° in benzene solution. It differs from *cis*-azobenzene in two important respects: (*a*) polarity measurements lead to an apparent moment of about 1·6 D. (in contrast to 3·0 D. for *cis*-azobenzene) and (*b*) illumination (daylight) causes only a very slight *increase* of dielectric constant (in contrast to the marked decrease already recorded for *cis*-azobenzene).

Hydrazobenzene and azoxybenzene do not appear to interact mutually under the conditions stated. The apparent moment observed for a mixture is of the order to be expected from that of each solute separately.

DURING the last few years Hodgson has several times indicated generally that he questions the existence of *cis*-azobenzene (cf. *J. Proc. R.I.C.*, 1947, 248; *Chem. and Ind.*, 1947, 774; 1948, 270, 428, 588). Recently a fuller exposition of his views has appeared (*J.*, 1948, 1097). Under the sub-title " A Critical Discussion of Some of the Properties of the Alleged *cis*-Azobenzene," he says (p. 1102) " . . . that a double compound of azoxybenzene and hydrazobenzene exhibits far more convincingly many of the reactions given by *cis*-[azobenzene]." This statement is the basis for the remainder of Hodgson's considerations.

The correctness of the underlying suggestion seems to us *a priori* unlikely. Among other measured physical properties, " *cis*-azobenzene " has a dipole moment in benzene solution of 3·0 D. units (Hartley and Le Fèvre, *J.*, 1939, 531). This is notably higher than the values for (ordinary) azoxybenzene (1·57 D., Calderbank and Le Fèvre, *J.*, 1948, 1949) or hydrazobenzene (1·79 D., present work) separately in the same solvent. Unless molecular compound formation takes place, therefore, it should be impossible for these two solutes together to simulate the *cis*-azobenzene with which we are familiar.

To investigate the last eventuality we have examined the dielectric constants and densities of two solutions containing equivalent quantities of azoxybenzene and hydrazobenzene.

EXPERIMENTAL.

These solutions had the following compositions: (*A*), 0·1527, 0·1678, and 92·023$_8$ g.; (*B*) 0·2982, 0·3256, and 83·857$_5$ g., of hydrazobenzene, azoxybenzene, and benzene respectively in each case. They were prepared, with exclusion of daylight, in brown glass bottles, from pure solutes and solvent which

had been stored over sodium wire. Measurements were commenced forthwith. Apparatus and technique were as noted in *J.*, 1948, 1949. Table I displays the results (determined relatively to benzene, see Table II).

TABLE I.

	$\varepsilon_{25°}$ (before exposure).	$\varepsilon_{25°}$ (calc.).	$\varepsilon_{25°}$ (after exposure).	$d_{4°}^{25°}$ (before exposure).	$d_{4°}^{25°}$ (calc.).	$d_{4°}^{25°}$ (after exposure).
Soln. (*A*)	2·2792	2·2795	2·2805	$0·8745_4$	0·8745	$0·8744_9$
Soln. (*B*)	2·2873	2·2873	2·2887	$0·8754_8$	0·8753	$0·8754_3$

Discussion.—Densities and dielectric constants of dilute solutions of graded dilution can frequently be represented by the equations: $d_{12} = d_2(1 + \beta w_1)$ and $\varepsilon_{12} = \varepsilon_2(1 + \alpha w_1)$, where w_1 is the weight fraction of the solute in the total mixture and α and β are constants (cf. Le Fèvre, "Dipole Moments," 2nd Edn., Methuen, 1948, pp. 39—41). From Le Fèvre and Calderbank's observations on azoxybenzene, and our own on hydrazobenzene, values for α and β appropriate to the present problem are available. By taking first one solute and then the other with the benzene, weight fractions may be ascribed to each of the three components in (*A*) or (*B*), and ε_{12} or d_{12} expressed as $\varepsilon_2(1 + \alpha w_1 + \alpha' w'_1)$ or $d_2(1 + \beta w_1 + \beta' w'_1)$ respectively (see Table I).

The closeness of the figures so obtained (cols. 3 and 6) to those from experiment (cols. 2 and 5) indicates that each solute has contributed independently. A direct test of Hodgson's suggestion may be made by treating our data as a determination of the dipole moment of "*cis*-azobenzene." Then w_1 would be the fraction: total solute/total solution. Table II shows our measurements set out in conventional form.

TABLE II.

$10^6 w_1$	0	3471	7384	Mean $\alpha\varepsilon_2$	= 1·97
$\varepsilon_{25°}$	2·2725	2·2792	2·2873	Mean β	= 0·257
$d_{4°}^{25°}$	0·87378	0·87454	0·87548	$_\infty P_1$	= 113·6 c.c.

whence, if $[R_L]_D = 59·2$ c.c. (Auwers, *Ber.*, 1938, **71**, 611), $\mu = 1·6_2$ D.

These results should be contrasted with those of Hartley and Le Fèvre (*loc. cit.*), *viz.*,

	$\alpha\varepsilon_2$.	β.	$_\infty P_1$ c.c.	μ, D.
cis-Azobenzene	6·05	0·221	255·3	3·0
trans- ,, ...	0·51	0·192	67·4	*ca.* 0

It is quite evident therefore that, in polar properties, the mixture of azoxy- and hydrazo-benzene does not resemble *either cis*- or *trans*-azobenzene.

An important characteristic of solutions of both the forms of azobenzene (Hartley and Le Fèvre, *loc. cit.*) is the action on them of daylight. Thus in sodium-dried benzene, the *same* equilibrium mixture can be produced from solutions initially containing only one of the separate varieties. Further, if a photo-equilibrated mixture is kept in the dark, it slowly becomes wholly of *trans*-content. Re-illumination can reproduce the equilibrium, etc. Because of the substantial differences (just quoted) in $\alpha\varepsilon_2$ for the *cis*- and *trans*-isomers, the processes can be detected easily and followed kinetically *via* the dielectric constants of the solutions. The same phenomena occur in solvents other than benzene (*e.g.*, "sodium-dry" ligroin, compare Hodgson, *loc. cit.*, p. 1102). For these reasons, therefore, we have submitted (*A*) and (*B*) to the test of exposure for 1 hour to sunlight, transferring them to clear glass flasks for the purpose. (At this stage it was obvious for the first time that the mixture was a pale yellow—quite unlike an azobenzene solution.) Redetermination then gave the figures in cols. 4 and 7 of Table I. The solutions became permanently deeper in colour. It will be seen that the dielectric constants *did not fall* as they should have done had the solute been authentic *cis*-azobenzene.

Two explanations are possible for the very slight increases actually obtained. The first is that some loss of benzene during the handling of the solutions from brown bottle to flask, through exposure, to condenser, etc., is unavoidable. The second is that we have found azoxybenzene of m. p. 38°, dissolved in dry benzene, itself to show a small increment of ε_{12} under parallel treatment. Thus a solution of $w_1 = 0·0504$ had $\varepsilon_{25°} = 2·2774$ before, and 2·2925 after, irradiation by direct sunlight. The increment—as with our mixtures of hydrazo- and azoxy-benzene—did not decay in the dark and was accompanied by a visible reddening. As to the latter, the literature records that ultra-violet light can bring about the formation from azoxybenzene of *o*-hydroxyazobenzene (Knipscheer, *Rec. Trav. chim.*, 1903, **22**, 1; cf. Cumming and Ferrier, *J.*, 1925, **127**, 2374, who report it also in benzene solution).

Melting Points of Mixtures of Hydrazo- and Azoxy-benzene.—An attempt to record a fusion point curve was abandoned when it became evident that approximately 50 : 50 mixtures became fluid at room temperatures. We therefore rubbed together accurately weighed equimolecular proportions and observed liquefaction to be complete at *ca.* 20° with considerable softening from 15° onward. Since the m. p.s concerned are hydrazobenzene, 131°, azoxybenzene, 35°, *cis*-azobenzene, 71°, and *trans*-azobenzene, 68°, the non-identity of Hodgson's "double compound" with *cis*-azobenzene is clear.

Dipole Moment of Hydrazobenzene.—This has been given by Audrieth, Nespital, and Ulich (*J. Amer. Chem. Soc.*, 1933, **55**, 673) as 1·53 D., and by Cowley and Partington (*J.*, 1933, 1252) as 1·66 D. We have made a re-determination in order to secure α and β values on a weight fraction basis for use in this paper:

$10^6 w_1$	0	3288·7	5753·5	12439
$\varepsilon_{25°}$	2·2725	2·2803	2·2864	2·2982
$d_{4°}^{25°}$	0·87378	0·87448	0·87504	0·87646

whence mean $\alpha\varepsilon_2 = 2·28$, mean $\beta = 0·247$, and $_\infty P_1 = 126·3$ c.c. If $[R_L]_D = 60$ c.c., then $\mu = 1·79$ D.

This moment is higher than either of the two previously recorded values, but since (*a*) we have merely assumed the refractivity of hydrazobenzene to be 2 × $R_{aniline}$ minus 2 × R_H, and (*b*) our solutions were only about one-tenth of the concentration of those of the other authors, we do not think the difference is significant at this stage; in any case it does not affect the main contention of this paper.

General Note.—Water would presumably be necessary to produce Hodgson's mixture from *trans*-azobenzene. We do not think it can enter seriously during our experiments. These always start with benzene standing over dry sodium. We have examined successfully, by the same technique, solutions of such solutes as carbon suboxide (*J.*, 1935, 1696) and diphenylketen (unpublished) which absorb water avidly. Further, if *cis*-azobenzene were as Hodgson suggests, during the (dark) reversion to the *trans*-form, water would be produced. Now the dielectric constant of the latter is *ca.* 80 and its effect on benzene is known (Williams, *J. Amer. Chem. Soc.*, 1930, **52**, 1831). Yet the final dielectric constants observed during the *cis*- to *trans*-transformation are equal to the ε values of freshly prepared *trans*-azobenzene solutions, and are evidently correct since they lead to polarisations close to the expected refraction figures (Hartley and Le Fèvre, *loc. cit.*). If, in fact, a half equivalent of water were present, the apparent polarisations would notably exceed the calculated molecular refractions. Further, we note that the literature mentions the reaction $Ph{\cdot}N_2O{\cdot}Ph + Ph{\cdot}NH{\cdot}NHPh = 2Ph{\cdot}N{:}NPh + H_2O$ as only occurring in the presence of reagents (such as alcoholic potash, Freundler, *Bull. Soc. chim.*, 1094, **31**, 459), not spontaneously in anhydrous media at ordinary temperatures. Effects of ultra-violet light may, of course, enter, but that the situation might not then be uncomplicated is suggested, *e.g.*, by the photolysis of azobenzene in aqueous alcohol mixtures (Fukushima, Horio, and Ohmori, *J. Soc. Chem. Ind. Japan*, 1932, **35**, 398) which, in the presence of zinc oxide and the absence of oxygen, produce hydrazobenzene quantitatively, but which, if oxygen is admitted, show no detectable change.

Finally, we mention that our views on other aspects of –N=N– stereochemistry raised by Hodgson's "reaffirmation" paper have been put forward in *Chemistry and Industry*, 1948, 158, 543, 732, 782, and in recent papers with Northcott (this vol., pp. 333, 944) and Wilson (*ibid.*, p. 1106).

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344. *Carbohydrate Sulphuric Esters. Part V. The Demonstration of Walden Inversion on Hydrolysis of Barium* 1 : 6-*Anhydro*-β-D-*Galactose* 2-*Sulphate.*

By R. B. DUFF.

2 : 3-1 : 6-Dianhydro-β-D-talose (I) has been obtained by the alkaline hydrolysis of *barium* 1 : 6-*anhydro*-β-D-*galactose* 2-*sulphate* (II). An improved method is described for the preparation of carbohydrate sulphates.

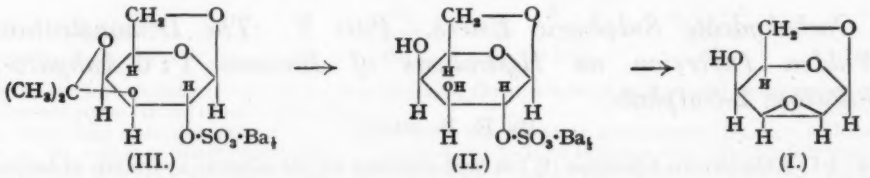
IN Parts I (Percival and Soutar, *J.*, 1940, 1475) and II (Duff and Percival, *J.*, 1941, 830) it was shown that barium methylhexoside sulphates (*e.g.*, barium α-methylglucoside sulphate) could be hydrolysed by aqueous alkali to give 3 : 6-anhydromethylhexosides. In Part III (Percival, *J.*, 1945, 119) carbohydrate sulphates (*e.g.*, barium methylglucofuranoside 3-sulphates) in which the sulphate residue was adjacent to a *trans*-hydroxyl group, were studied. It was expected that ethylene oxide anhydrides would be formed on hydrolysis with aqueous alkali and that these would give recognisable products on complete hydrolysis. This was not realised and the conclusion was reached that for sulphates as distinct from toluene-*p*-sulphonates, ethylene oxide rings were not formed on alkaline hydrolysis, but that 3 : 6-anhydro-ring formation, together with the straightforward removal of the sulphate group, occurred.

In Part IV (Duff and Percival, *J.*, 1947, 1675), however, it was established that barium 3-methyl 1 : 2-*iso*propylidene glucofuranose 6-sulphate readily gave 3-methyl 1 : 2-*iso*propylidene 5 : 6-anhydroglucofuranose on treatment with sodium methoxide in methanol. This barium salt was chosen as it was substituted at $C_{(3)}$ and thus the formation of a 3 : 6-anhydride either directly or through the 5 : 6-anhydride (cf. Seebeck, Meyer, and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1142) was prevented. Further, the material was soluble in organic solvents, and previous difficulties due to decomposition of the ethylene oxide anhydride in aqueous alkali were avoided. As the sulphate residue was attached to $C_{(6)}$, no Walden inversion could be demonstrated with the above material, and attempts to prepare anhydrides, with inversion, from barium 4 : 6-benzylidene α-methylglucoside sulphate and barium 6-methyl β-methylgalactopyranoside 2-sulphate were unsuccessful. In the latter case treatment with sodium methoxide in methanol resulted in extensive decomposition with the production of reducing substances. It was evident that the adjacent sulphate group rendered the glycosidic methoxyl labile to alkali, as in the similar case of the hydrolysis of a 2-hydroxyethanesulphonic acid glycoside recorded by Helferich and Schnorr (*Annalen*, 1941, **547**, 201).

Therefore, in the present work, attention was directed to the synthesis of a carbohydrate sulphate with a grouping on $C_{(1)}$ more stable to alkali than in the previous case. The compound chosen, *barium* 1 : 6-*anhydro*-β-D-*galactose* 2-*sulphate* (II) was obtained from *barium* 3 : 4-iso*propylidene* 1 : 6-*anhydro*-β-D-*galactose* 2-*sulphate* (III), formed on sulphating 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose which was obtained by the method of Hann and Hudson (*J. Amer. Chem. Soc.*, 1942, **64**, 2435). Although (II) was soluble only to a limited extent in methanol, it reacted smoothly with a solution (8%) of sodium in dry methanol at 55° to give 1 : 6-2 : 3-dianhydro-β-D-talose in 36% yield. It was necessary to recrystallise the dianhydride five times to obtain physical and analytical data in agreement with the values given by James *et al.* (*J.*, 1946, 625) and to give no depression in melting point when mixed with an authentic specimen prepared from 1 : 6-anhydro-β-D-galactose 2-toluene-*p*-sulphonate.

When (II) was heated at 100° for 24 hours with methanolic sodium methoxide, some charring took place and it was necessary to isolate the partly methylated products by continuous extraction. The methoxyl content (11·5—13·4%) of these fractions indicated an impure monomethyl 1 : 6-anhydrohexose (Calc.: 14·35%). That the latter was probably 2-methyl 1 : 6-anhydro-β-D-galactose was indicated by paper chromatography (Partridge, *Nature*, 1946, **158**, 270) in which comparable R_G values were obtained from the above syrups and from authentic 2-methyl 1 : 6-anhydrogalactose after hydrolysis with acid to the corresponding 2-methyl hexose. No evidence was obtained of the presence of 3-methyl 1 : 6-anhydro-β-idose which could also be obtained from the intermediate dianhydrotalose.

An attempt was made to prepare the crystalline 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose from a portion of the syrup. Crystalline material was obtained by cooling with ether and solid carbon dioxide, but attempts to raise the melting point did not succeed owing to difficulty in finding a suitable solvent for recrystallisation and to the small yield. The melting point (30°) of the product was not depressed on mixing with an authentic specimen (m. p. 37°).



On hydrolysis with aqueous alkali, (II) would be expected to give 2 : 3-1 : 6-dianhydro-β-D-talose (I), followed by 1 : 6-anhydro-β-D-galactose and/or 1 : 6-anhydro-β-D-idose. Paper chromatography was used to investigate this reaction, the material obtained by alkaline hydrolysis of (II) and of an authentic specimen of 2 : 3-1 : 6-dianhydro-β-D-talose being hydrolysed to the corresponding sugars by dilute sulphuric acid. In both cases spots with an R_G value corresponding to that of galactose were observed, but evidence of the formation of idose could not be obtained.

Baumgarten's pyridine sulphuric anhydride, $C_5H_5N<\begin{matrix}O\\SO_2\end{matrix}$ (*Ber.*, 1926, **59**, 1166, 1978) was used as a sulphating agent in this work. The method used previously involved adding a solution of chlorosulphonic acid in chloroform to a pyridine solution of the sugar at −15°. The mixture was slowly diluted with water, excess sulphuric acid removed by barium carbonate, and chloride ions by silver sulphate.

Pyridine sulphuric anhydride has not previously been used as a sulphating agent for carbohydrates. There is usually little heat evolved on mixing the reagent with a pyridine solution of the carbohydrate derivative, and the temperature can be maintained in the range 15—50°. The mixture is diluted with water, free sulphuric acid removed by barium carbonate, and the filtrate evaporated, to give the pure barium salt of the sugar sulphate. The use of large volumes of silver sulphate solution is thus avoided. When glucose was converted into barium glucose monosulphate by this method, a yield of 96% was obtained, which compares favourably with values previously quoted.

As noted (Percival and Duff, *Nature*, 1946, **158**, 29), realisation of Walden inversion on hydrolysis of carbohydrate sulphates in the laboratory makes it necessary to envisage the interconversion of sugars in nature *via* the ethereal sulphates and ethylene oxide anhydrides.

A mechanism for the conversion of glucose into galactose in animals by way of the 5-phosphate has been indicated by Peat ("Advances in Carbohydrate Chemistry," 1946, II, 54), and the

occurrence of 4-sulphates of carbohydrates in nature may have significance for a similar mechanism in plants.

Experimental.

Barium 3 : 4-iso*Propylidene* 1 : 6-*Anhydro*-β-D-*galactose* 2-*Sulphate*.—3 : 4-*iso*Propylidene 1 : 6-anhydro-β-D-galactose (7·73 g.) was dissolved in dry redistilled pyridine (150 c.c.), and pyridine sulphuric anhydride (20 g., 3·3 mols.) added with shaking. The mixture was stirred with a vigorous current of nitrogen and heated at 50° for 2½ hours, and then poured, with stirring, into excess of a suspension of barium carbonate in water. After removal of barium salts by filtration, the solution was evaporated to dryness at 30°/15 mm. in the presence of a little barium carbonate, and the residue dried with alcohol and benzene. Unchanged material was removed by four extractions with hot ethyl acetate. Dissolution in water, followed by filtration and evaporation to dryness, gave the pure *barium* salt (11·9 g., 89%), $[\alpha]_D^{15°}$ −35·0° (*c*, 1·9 in water) (Found: Ba, 20·2; SO_4, 28·4; acetone, 14·2. $C_9H_{13}O_8SBa_{\frac{1}{2}}$ requires Ba, 20·8; SO_4, 29·1; acetone, 16·6%).

Barium 1 : 6-*Anhydro*-β-D-*galactose* 2-*Sulphate*.—Barium 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose 2-sulphate (11·6 g.) in water (70 c.c.) was treated with 0·2N-sulphuric acid (300 c.c.), and the mixture was kept at 37° for 48 hours. Sulphuric acid was removed as usual and the *product* isolated by evaporation at 30°/15 mm. (8·9 g.); $[\alpha]_D^{15°}$ −10·6° (*c*, 1·2 in water) (Found: Ba, 22·3; SO_4, 30·7; acetone, 0·2. $C_6H_9O_8SBa_{\frac{1}{2}}$ requires Ba, 22·2; SO_4, 31·0%).

Hydrolysis of Barium 1 : 6-*Anhydro*-β-D-*galactose* 2-*Sulphate with Sodium Methoxide*.—The preceding barium salt (3·6 g.) was heated under reflux for 30 minutes with anhydrous methanol (100 c.c.) but did not dissolve completely. Sodium (5·0 g.), dissolved in methanol (60 c.c.), was added to the cooled suspension, and the mixture heated at 55° for 2 hours while being stirred with a current of nitrogen. Insoluble salts were removed by centrifugation, and the supernatant liquor neutralised (phenolphthalein) with carbon dioxide, water being added from time to time to dissolve precipitated sodium carbonate. The solution was extracted with chloroform (ten times), the combined extracts (1 l.) were dried (Na_2SO_4), and the filtered solution was evaporated at 35°/15 mm. The dry residue was extracted three times with ethyl acetate, and the combined extracts were evaporated to small bulk. Light petroleum (b. p. 40—60°) was added, and the solution decanted from precipitated gummy material. Further addition of light petroleum gave a cloudy liquid which deposited needles (0·6 g., 36%), m. p. 122—124°, when kept at 0°. Purified by recrystallisation five times from ethyl acetate and light petroleum (b. p. 40—60°), the product had m. p. 131—132°, not altered on admixture with authentic 1 : 6-2 : 3-dianhydro-β-D-talose prepared from 1 : 6-anhydro-β-D-galactose 2-toluene-*p*-sulphonate (Found: C, 50·2; H, 5·8. Calc. for $C_6H_8O_4$: C, 50·0; H, 5·6%), $[\alpha]_D^{18°}$ −88·0° (*c*, 1·4 in water). James *et al.* (*loc. cit.*) give m. p. 132° and $[\alpha]_D^{20°}$ −88° (*c*, 0·76 in water).

In another experiment the barium salt (1·97 g.) was heated under reflux overnight with a solution of sodium (1·5 g.) in methanol (40 c.c.). The solution was neutralised with carbon dioxide, diluted with water, and transferred to a continuous-extraction apparatus. Extraction with light petroleum (b. p. 40—60°) for 4 days, followed by removal of the solvent, gave a syrup (0·026 g.) (Found: OMe, 11·5%). A similar operation with the use of benzene gave a pale syrup (0·109 g.) (Found: OMe, 13·4%). Finally the solution was extracted continuously with chloroform for 4 days, to give a clear syrup (0·195 g.) (Found: OMe, 12·7%). The material extracted as above was thought to be an impure methyl anhydrohexose (Calc.: OMe, 14·35%), and the fractions were further investigated on the paper chromatogram (Partridge, *loc. cit.*) with tetramethyl glucose as standard. In a typical experiment the syrup (from the benzene extract) gave spots of R_G 0·094 and 0·214. The syrup (from the chloroform extract) gave spots of R_G 0·094 and 0·212. 2-Methyl galactose containing a little galactose (from 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose) gave spots of R_G 0·093 and 0·215.

In the above experiments the 1 : 6-anhydrides were hydrolysed to the sugars by treatment for 18 hours at 100° in a sealed tube with 2N-sulphuric acid. Acid was then removed with barium carbonate, the mixture centrifuged, and the supernatant liquor spotted on the chromatogram. The R_G values found agree reasonably well with those given by Brown *et al.* (*Nature*, 1948, **161**, 720) for galactose (R_G 0·075) and 2-methyl galactose (R_G 0·205). In a further attempt to characterise the syrup the material (0·090 g.) from the chloroform extract was converted into the *iso*propylidene compound by the method of Ohle and Thiel (*Ber.*, 1933, **66**, 525) as modified by McPhillamy and Elderfield (*J. Org. Chem.*, 1939, **4**, 150). The product was distilled at 110—120° (bath-temperature)/0·01 mm.; it crystallised on freezing with solid carbon dioxide–ether; yield, 0·030 g.; m. p. 30°, unchanged on mixing with authentic 2-methyl 3 : 4-*iso*propylidene 1 : 6-anhydro-β-D-galactose, m. p. 37°.

Hydrolysis of Barium 1 : 6-*Anhydro*-β-D-*galactose* 2-*Sulphate with Aqueous Alkali*.—The barium salt (0·06 g.) was heated for 18 hours at 100° in a sealed tube with barium hydroxide (1·04 g.) and water (2 c.c.). The solution was neutralised (phenolphthalein) with carbon dioxide, the filtrate taken to dryness at 30°/15 mm., and the syrupy residue heated for 18 hours at 100° with 2N-sulphuric acid (5 c.c.). After removal of sulphuric acid with barium carbonate and evaporation of the filtrate, the residue was dissolved in a few drops of water and examined by paper chromatogram.

1 : 6-2 : 3-Dianhydro-β-D-talose (0·043 g.) was treated similarly to provide a standard. In both cases spots with R_G 0·075, corresponding to galactose, were obtained, and idose, if formed, was not separated.

Barium Glucose Monosulphate.—Glucose (10 g.) was mixed at room temperature with dry pyridine (150 c.c.) and pyridine sulphuric anhydride (13·7 g., 1·5 mol.) (prep.: "Inorganic Syntheses," 1946, **11**, 173, McGraw-Hill, New York). The mixture was shaken at intervals for 4 hours and kept overnight in a stoppered flask; sulphuric acid was then removed by adding water (200 c.c.) and barium carbonate (50 g.) with agitation. The solution was heated at 50° for 2 hours to complete neutralisation, and excess of barium salts removed by filtration. The filtrate was evaporated to small bulk at 35°/15 mm. and poured into absolute alcohol (1 l.) with mechanical stirring, and the precipitate was dried *in vacuo* over phosphoric oxide; yield, 17·5 g. (96%), $[\alpha]_D^{24°}$ +32° (*c*, 2·9 in water at equilibrium) (Found: Ba, 20·5; SO_4, 29·8. Calc. for $C_6H_{11}O_9SBa_{\frac{1}{2}}$: Ba, 21·0; SO_4, 29·3%).

2-Methyl 3 : 4-iso*Propylidene* 1 : 6-*Anhydro-β-*D*-galactose.*—3 : 4-*iso*Propylidene 1 : 6-anhydro-β-D-galactose was methylated three times with Purdie's reagents. The product had m. p. 37°.

The author wishes to express his warm thanks to Dr. E. G. V. Percival for his interest in this work. Thanks are also expressed to Dr. W. V. Thorpe of the Physiology Department, Birmingham University, who drew the author's attention to the existence of pyridine sulphuric anhydride, and to Edinburgh University for the award of an I.C.I. Fellowship.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, January 26th,* 1949].

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345. *Studies on Seed Mucilages. Part III. Examination of a Polysaccharide extracted from the Seeds of* Plantago ovata *Forsk.*

By R. A. LAIDLAW and E. G. V. PERCIVAL.

Extraction of the seeds with cold water yielded a polysaccharide (PI) of equivalent *ca.* 700, giving on analysis uronic anhydride (20%), pentosan (52%), and methylpentosan (18%). Further extraction with hot water gave PII, equivalent *ca.* 4,000, containing *ca.* 3% of uronic anhydride.

Hydrolysis of PI gave D-xylose (*ca.* 46%), L-arabinose (*ca.* 7%), and an aldobiuronic acid (40%) shown to be 2-D-galacturonosido-L-rhamnose, and an insoluble residue (2%). From PII, L-arabinose (*ca.* 14%) and D-xylose (*ca.* 80%) were isolated, along with an insoluble residue (3%).

Acetylation, followed by deactylation and methylation of PI and subsequent fractional precipitation, gave materials *A* (82%) and *B* (18%). Methanolysis of *A* yielded trimethyl methyl-D-xylopyranosides (6 parts), trimethyl methyl-L-arabofuranosides (1 part), 2 : 4-dimethyl methyl-D-xylopyranosides (1 part), 3-methyl methyl-D-xylopyranosides (4 parts), 2-methyl methyl-D-xylopyranosides (1 part), and methyl-D-xylopyranosides (2 parts). *B* gave similar fractions, except that double the proportion of 2 : 4-dimethyl methyl-D-xylosides was present at the expense of monomethyl methyl-D-xylosides.

The aldobiuronic acid is probably a component of a polyuronide associated with a neutral polysaccharide rather than an integral part of a single acidic polysaccharide.

ANDERSON and FIREMAN (*J. Biol. Chem.*, 1935, **109**, 437) studied the mucilage extracted from the so-called light "Psyllium" seeds and suggested that it might be represented as a chain of D-xylose units, varying in number between 7 and 36, to which was attached an aldobiuronic acid formed by the union of L-arabinose and D-galacturonic acid.

In Part II of this series (*J.*, 1942, 58) the results of an investigation on the mucilage of dark "Psyllium" seeds (*Plantago arenaria*) were discussed. Hydrolysis of this mucilage gave D-xylose, L-arabinose, D-galactose, and D-galacturonic acid. It was evident that the polysaccharide isolated from *P. arenaria* differed in composition from that described by Anderson and Fireman (*loc. cit.*) and, since light "Psyllium" or ispaghula has been identified with *Plantago ovata* Forsk (Skryme, *Quart. J. Pharm.*, 1935, **8**, 161), it was decided to investigate the mucilages of this species, especially in view of the introduction of improved methods for the separation of mixtures of methylated sugars.

Anderson and Fireman (*loc. cit.*) pointed out that the composition of their extract varied with the conditions of extraction. Similar variations were found in our experiments; extraction with cold water gave a product (PI) having a higher uronic acid content and a lower pentosan content than the polysaccharide (PII) isolated by extracting the residue at 90—95°. The polysaccharide obtained by extraction with cold water and precipitation in acidified alcohol (PI) had an apparent equivalent weight of *ca.* 700, giving on analysis uronic anhydride (20%), pentosan (52%), and methyl pentosan (18%), whereas the fraction isolated from the residual seeds by water at 90—95° (PII) had an apparent equivalent weight of *ca.* 4000, and contained uronic anhydride (*ca.* 3%) and pentosan (*ca.* 90%).

The mucilages examined were thus very probably mixtures of at least two distinct polysaccharides: one, readily soluble in water, containing a high proportion of uronic acid units, and the other neutral. This may be compared with gum tragacanth (Jones and Smith, *J.*, 1945, 739, 749) which was shown to be a mixture of a polyuronide, a neutral polysaccharide, and another fraction, probably a sterol glucoside, and with pectin, which is a complex mixture of pectic acid, associated with an araban and a galactan.

Hydrolysis of PI with 3% oxalic acid gave, after neutralisation with barium carbonate, a complex barium salt *X* (44%) and a syrup containing D-xylose and L-arabinose. The arabinose, identified and estimated as the diphenylhydrazone, constituted some 6% of the original mucilage. More drastic hydrolysis of *X* gave a degraded barium salt *Y* (67·5% of *X*) and a reducing syrup (32·5%) which was chiefly D-xylose but contained L-arabinose (3% of *X* or 1·4% of the original

hydrolysed PI). Further hydrolysis of *Y* with sulphuric acid afforded rhamnose, along with traces of D-xylose and L-arabinose, and a barium salt *Z*, which, on drastic hydrolysis, yielded L-rhamnose and D-galacturonic acid. Anderson and Fireman (*loc. cit.*) did not report the presence of L-rhamnose among the hydrolysis products of their material, but the estimated proportions of L-rhamnose (18%) and D-galacturonic acid (20%) would agree with the assumption that a D-galacturonosido-L-rhamnose formed a building unit in PI.

As with the mucilage from *Plantago lanceolata* (Part I, *J.*, 1940, 1501), acetylation gave products which could be separated into fractions, soluble and insoluble in acetone–chloroform (1 : 1). The general properties of these acetates were unchanged on further acetylation, and methylation of the crude acetates and of the various fractions gave products which were essentially similar, apart from their specific viscosities, and, presumably, molecular sizes. Table I shows the results of fractional precipitations of a number of methylated products.

TABLE I.

Fraction.	Yield, %.	OMe, %.	$[\alpha]_D^{15°}$ ($CHCl_3$).	$\eta_{sp}^{20°}/c'$.
1	63	33·5	−121°	149
2	24	34·7	−123	97
3	13	34·5	−122	62
A	82	34·0	−121	145
B	18	35·0	−123	73

Where c' is the concentration in g.-mols. of methylated anhydro-xylose residues per l.

Methanolysis of A (32 g.) and B (7 g.) gave a mixture of methylated sugar glycosides, which were separated by solvent extraction with light petroleum (Brown and Jones, *J.*, 1947, 1344) and with chloroform, and by distillation, to yield the fractions discussed below.

The most highly methylated fractions were mainly trimethyl methylxylopyranosides, which gave on hydrolysis crystalline trimethyl α-D-xylopyranose which yielded a crystalline *anilide*. Polarimetric observations indicated, however, that this sugar was not the only component of the fraction, although no separation could be achieved by chromatography on alumina (Jones, *J.*, 1944, 333). After the removal of several crops of crystalline trimethyl xylose from the hydrolysed fraction over a prolonged period the remaining syrupy material was oxidised and the resulting lactones distilled. Crystalline 2 : 3 : 5-trimethyl L-arabonamide was prepared from the first fraction, and from the equilibrium rotations of the lactone fractions it was estimated that the fully methylated material consisted of a mixture of trimethyl methyl-D-xylopyranosides (85%) and trimethyl methyl-L-arabofuranosides (15%).

The fractions of methoxyl content corresponding to dimethyl methylpentosides gave on hydrolysis crystalline 2 : 4-dimethyl β-D-xylose, which was oxidised to the syrupy 2 : 4-dimethyl D-xylopyranolactone (Barker, Hirst, and Jones, *J.*, 1946, 783). The derived amide gave a negative Weerman test, and oxidation of the free sugar with periodic acid (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1473) gave no formaldehyde. Crystalline 2 : 4-dimethyl D-xylose anilide was also isolated. These findings were confirmed by mixed-m. p. determinations on the free sugar (kindly supplied by Dr. J. K. N. Jones) and on the anilide. Finally, by means of the paper chromatogram it was shown that 2 : 4-dimethyl D-xylose was the only sugar present. The fraction, therefore, consisted entirely of 2 : 4-dimethyl methyl-D-xylopyranosides.

The fraction corresponding to monomethyl methylpentosides, yielded, on methylation followed by hydrolysis, crystalline trimethyl xylopyranose as the sole product. Periodate oxidation of the free sugar gave formaldehyde in 27% yield, which seemed to indicate that some 4-methyl xylose was present. It may be recalled, however, that Bell (*J.*, 1948, 992) has shown that this is not necessarily a true estimate since several sugars, *e.g.*, 3-methyl glucose, 2 : 4-dimethyl galactose, 3 : 4-dimethyl mannose, etc., gave a yield of formaldehyde which was by no means quantitative. Oxidation of the free sugars gave a crystalline lactone admixed with syrup. The crystalline material, m. p. 93—94°, behaved as a γ-lactone and the sodium salt of the acid on periodate oxidation gave a high yield of formaldehyde, indicating the absence of a methoxyl residue on $C_{(4)}$. This was confirmed by the fact that the free sugar gave methyl-furanosides as well as methylpyranosides on treatment with cold methanolic hydrogen chloride. The amide derived from the crystalline lactone gave a positive Weerman test, showing that $C_{(3)}$ was unsubstituted; further, the free sugar yielded a monomethyl xylosazone. The sugar was therefore, 3-methyl xylose. In confirmation of this, the crystalline *lactone* and *anilide* were identical with products prepared from synthetic 3-methyl D-xylose (Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331). The main constituent of the hydrolysed syrup was, therefore,

3-methyl D-xylopyranose which yielded 3-*methyl* D-*xylonolactone*, 3-*methyl xylonamide*, 3-*methyl xylose anilide*, and 3-*methyl xylosazone*.

The main bulk of the monomethyl xylose fraction was, therefore, 3-methyl methyl-D-xylopyranosides. There were, however, several indications that this was not the only constituent. The syrupy fraction of the lactone on hydrolysis and subsequent periodate oxidation yielded some formaldehyde, indicating the presence of a free hydroxyl group on $C_{(4)}$. The amide prepared from this syrupy lactone, however, gave a negative Weerman test. This would seem to indicate 2-methyl xylose as the parent sugar. Further, after treatment of the free sugars with aniline and removal of as much 3-methyl xylose anilide as possible, followed by hydrolysis and oxidation with nitric acid, the product appeared to be an optically active dihydroxymethoxyglutaric ester ($[\alpha]_D^{18°} + 39°$ in methanol) which must have been unsymmetrical and could have been derived from either 2- or 4-methyl xylose. That the former possibility was correct was confirmed by comparison, on a paper chromatogram, with an authentic specimen. The minor component (20%) was, therefore, 2-methyl methylxylopyranosides which yielded 2-methyl D-xylose, 2-methyl D-xylonolactone, 2-methyl D-xylonamide, and *dihydroxy-2-methoxy-xyloglutaric* ester.

The remaining glycosides contained a high proportion of free methylxylosides, with some 3-methyl methylxylosides. The presence of the former was established by the isolation of crystalline D-xylose and β-methylxyloside, and by the formation of dibenzylidene xylose dimethyl acetal (Breddy and Jones, *J.*, 1945, 738); presence of the 3-methyl methylxylosides was confirmed by isolation of the crystalline anilide. These findings were further confirmed by means of the paper chromatogram.

It was estimated that the methanolysis products of *A* were composed of trimethyl methyl-D-xylosides (6 parts), trimethyl methyl-L-arabinosides (1 part), 2 : 4-dimethyl methyl-D-xylosides (1 part), 3-methyl methyl-D-xylosides (4 parts), 2-methyl methyl-D-xylosides (1 part), and methyl-D-xylosides (2 parts).

In the polysaccharide, therefore, the following residues must be linked as shown,

$$X1;\ A1;\ \overset{1}{X}3;\ 4\overset{1}{X}2;\ 4\overset{1}{X}3;\ 4\overset{1}{X}\underset{3}{2}$$

(X = D-xylopyranose)
(A = L-arabofuranose)

although no information is available at this stage to indicate how these residues are linked in the mucilage molecule.

The proportion of unsubstituted xylose units is too high to be accounted for by incomplete methylation of the mucilage, repeated attempts to raise the methoxyl content of the methylated polysaccharide having failed, even using the thallium method. A certain amount of demethylation has, however, been shown to occur during methanolysis. Thus, a sample of 3-methyl D-xylopyranose, on methanolysis followed by hydrolysis under the conditions used for the methylated polysaccharide, was shown to have been demethylated to the extent of *ca.* 5%, although trimethyl xylopyranose showed no appreciable demethylation when similarly treated. It is highly improbable therefore that the free xylose could have been formed in this way. In any event, the high proportion of end-groups found necessitates the presence of a relatively large proportion of free xylose units in the methylated molecule. Thus, a hypothetical repeating unit such as is indicated would give on hydrolysis a mixture of glycosides in the following

$$\begin{array}{ccccccccccc} & & & & X & & & & & & \\ & & & & 1 & & & & & & \\ & & & & 2 & & & & & & \\ \ldots & & 3X1 & & 3X1 & & 4X1 & & 4X1 & 4X1 & 3X1 \ldots \\ & & 4 & & & & 2 & & 2 & 2 & 4 \\ & & 1 & & & & 1 & & 1 & 1 & 1 \\ & & X & & & & X & & X & X & A \end{array}$$

proportions: trimethyl methylxylosides, 44·7; trimethyl methylarabinosides, 8·9; dimethyl methylxylosides, 8·3; monomethyl methylxylosides, 30·9; methylxylosides, 7·1%, whereas the quantities estimated were 43·5, 7·5, 6·5, 32·5, and 10%, respectively. Such a structure, however, should yield on oxidation with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427) 5 moles of formic acid, *i.e.*, 0·00311 mol./g. The value found in practice for a specimen of the polysaccharide regenerated from the acetate was 0·00227 mol./g. Although this result is in agreement with the postulation of a highly branched structure, fewer terminal xylopyranose residues are indicated by the periodate method than by the methylation process, and further work will be necessary to explain this divergence.

The results obtained from methylated polysaccharide fraction B were closely similar, except that the proportion of 2 : 4-dimethyl methylxylosides appeared to be doubled at the expense of the monomethyl methylxylosides.

A comparison of the results reported here for *P. ovata* with those previously recorded for *P. arenaria* (Part II, *loc. cit.*) reveals that the anilide, m. p. 170°, $[\alpha]_D^{11°}$ −74°, isolated on that occasion was undoubtedly 2 : 4-dimethyl xylose anilide, and not a derivative of arabinose as suggested, and the L-arabinose in *P. arenaria* mucilage may possibly also occur as an end-group. No 3: 4-dimethyl xylose was detected in the present series of experiments, but this is not surprising, for the reasons outlined in Part IV. Apart from the confusion which has existed about the isolation of 3 : 4-dimethyl xylose from the methylated seed mucilages previously studied (Parts I and II, *loc. cit.*), the main points of difference between *P. ovata* and the others of this class are the absence of D-galactose in the present instance and the existence of a high proportion of xylose units with a free hydroxyl group on $C_{(3)}$. *P. arenaria* mucilage was shown to contain no L-rhamnose, although, on hydrolysis of the methylated mucilage, a high proportion (23%) of 2-methyl xylose was isolated.

The barium aldobiuronate *Z* was methylated and hydrolysed to yield 3 : 4-dimethyl L-rhamnose and 2 : 3 : 4-trimethyl D-galacturonic acid, identified as the crystalline methyl 2 : 3 : 4-trimethyl mucate. There is, as yet, no proof as to whether the linkage between these two units is α- or β- in form, but the highly positive rotation of the barium salt of the acid and of its methylated derivative would appear to indicate that it is α.

The aldobiuronic acid was, therefore, 2-D-galacturonosido-L-rhamnose, identical with that isolated from flax-seed mucilage (Tipson, Christman, and Levene, *J. Biol. Chem.*, 1939, **128**, 609) and slippery-elm mucilage (Gill, Hirst, and Jones, *J.*, 1939, 1469).

It will have been noted that no methylated uronic acid or methyl pentose derivatives were isolated from the hydrolysis products of the methylated mucilage. Analysis of the acetylated polysaccharide, however, showed that such residues were absent. Evidence was also obtained by direct methylation that PI could be separated into an insoluble, partly methylated derivative, devoid of uronic acid, and a soluble product still containing uronic acid residues. These observations lend support to the view already expressed that the aldobiuronic acid residue is a constituent of an associated polyuronide, although a direct separation has not so far been achieved.

Experimental.

All evaporations were conducted under diminished pressure unless otherwise stated; the temperatures recorded are bath-temperatures; the concentration of rotation solutions is 1% unless otherwise stated.

Preparation of PI.—*P. ovata* Seeds (150 g.) were steeped in water (3 l.) at 15° for 48 hours, with occasional stirring. The highly viscous solution was separated by filtration through muslin, and the residue washed with water. The solution was added to ethanol (3 volumes), and the fibrous product dried with ethanol and ether, and in a vacuum over phosphoric oxide. Yield, *ca.* 1·5% [Found: Ash, 5·2%; (as sulphate, 7·7%)]. Precipitation with acidified ethanol (20 c.c. of concentrated hydrochloric acid per l.) yielded an almost ash-free polysaccharide (1·5%) (Found: equiv., by titration, *ca.* 700; uronic anhydride, 20·3%; pentosan, 52%; methylpentosan, 18%).

Hydrolysis of PI *with* 3% *Oxalic Acid.*—The dry mucilage (2·001 g.) was hydrolysed with 3% oxalic acid (100 c.c.) at 100°. $[\alpha]_D^{18°}$ were +31° (1 hour), +47° (2 hours), +56° (3 hours), +60·5° (4 hours), +43·5° (52 hours, constant). A larger quantity (18·38 g.) was hydrolysed at 100° as above for 6 hours (final $[\alpha]_D^{18°}$ +65°). The insoluble residue (0·33 g.) was removed, and the filtrate neutralised with barium carbonate, filtered, and evaporated to 100 c.c. After removal of a trace of inorganic material, the solution was poured into ethanol (2 l.), to give a white barium salt (*X*; 7·00 g.). Removal of the ethanol yielded a reducing syrup (*S*; 8·71 g.).

S partly crystallised, to yield D-xylose (5·77 g.), which was separated by trituration with glacial acetic acid. The crystalline material had m. p. 139—141°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{18°}$ +79° (initial value), +74° (10 minutes), +26° (80 minutes), +20° (4 hours, constant) in water, and yielded xylosazone, m. p. and mixed m. p. 157—159°.

The acetic acid washings were evaporated to give a reducing syrup (S_1) (2·90 g.), $[\alpha]_D^{18°}$ +34° in water (Found: methyl pentose, 2·4%). This syrup (0·353 g.) yielded L-arabinose diphenylhydrazone (0·231 g.), m. p. 190°, unchanged on admixture with an authentic specimen, corresponding to an arabinose content of 43·8%, or 6·1% of anhydro-arabinose in the mucilage. No galactose phenylmethylhydrazone could be obtained from S_1. After several days, S_1 crystallised to yield further quantities of D-xylose, identified as above.

Investigation of the Insoluble Residue.—The combined residues from several experiments (1·30 g.) with 72% sulphuric acid (Monier-Williams, *J.*, 1921, 803) yielded a reducing syrup (0·28 g.), with some apparently unchanged material (0·80 g.). Glucose was the only sugar identified on the paper chromatogram.

Acetylation. (*a*) PI (20 g.) was dispersed in pyridine (200 c.c.), and acetic anhydride (170 c.c.) added slowly with continuous shaking. The mixture was then heated at 100° for 3 hours and kept for a further 72 hours at room temperature. The acetate was precipitated by pouring into a large excess of water, filtered off, and washed in running water for 48 hours, to yield a white fibrous product [25 g.;

$\eta_{sp}^{20°}/c'$, 95·2 (*m*-cresol; *c'* as g.-mols. of acetylated anhydroxylose per l.); Ac, 35·0%], which was extracted with boiling acetone–chloroform (1 : 1) to give a soluble acetate (60%), $[\alpha]_D^{15°}$ −94° (in acetone) [Ac, 43·6%; $\eta_{sp}^{20°}/c'$, 88·6 (*m*-cresol)], and an insoluble acetate (40%) (Ac, 20·1%).

(*b*) PI (10 g.) with pyridine (150 c.c.) and acetic anhydride (100 c.c.) as above yielded a white fibrous product (13 g.; Ac, 29·1%). This gave a soluble acetate (40%), $[\alpha]_D^{13°}$ −65° (in chloroform) [Ac, 36·8%; $\eta_{sp}^{20°}/c'$, 188·1 (*m*-cresol)], and an insoluble fraction (60%) [Ac, 23·4%; $\eta_{sp}^{20°}/c'$, 161·6 (*m*-cresol)].

Methylation. Crude acetate from (*a*) above was methylated directly in three operations with dimethyl sulphate and sodium hydroxide as described by Mullan and Percival (Part I, *loc. cit.*) without extraction with solvents. This yielded a methylated polysaccharide (11·7 g.) {OMe, 35·3%; $[\alpha]_D^{14°}$ −126° (in chloroform); $\eta_{sp}^{20°}/c'$, 140 (*m*-cresol); these figures were unchanged on a further methylation}. Crude acetate from (*b*) above gave an almost identical product. The soluble acetate (3½ g.) from (*b*) yielded a methylated compound (1·1 g.) {OMe, 33·8%; $[\alpha]_D^{15°}$ −124° (in chloroform); $\eta_{sp}^{20°}/c'$, 152·0 (*m*-cresol)}. The insoluble acetate (5·5 g.) from (*b*) gave a methylated product (2·0 g.) {OMe, 36·4%; $[\alpha]_D^{15°}$ −127° (in chloroform); $\eta_{sp}^{20°}/c'$, 97·4 (*m*-cresol)}. To crude methylated product from (*b*) (1 g.) in chloroform (50 c.c.) was added thallous ethoxide (1 g.) in chloroform (10 c.c.); the mixture was evaporated to dryness, and the powdered residue heated with methyl iodide (50 c.c.) under reflux until a sample of the yellow solid gave no reaction with phenolphthalein (8 days); after filtration, the solvent was removed, and the residue exhaustively extracted with chloroform; the extracts were concentrated to small volume, and the methylated polysaccharide was precipitated by light petroleum {Found: OMe, 33·7%; $[\alpha]_D^{13°}$ −126° in chloroform; $\eta_{sp}^{20°}/c'$, 88·2 (*m*-cresol)}; this appeared to indicate that the polysaccharide was originally fully methylated.

The crude methylated polysaccharide from (*b*) was fractionated from chloroform solution to give the fractions listed in Table I.

The uronic acid contents of the various acetate fractions were *ca.* 2·5% (average), indicating considerable loss of uronic acid units during acetylation; the uronic acid contents of the various methylated materials were too small to determine accurately.

Direct Methylation of PI.—The polysaccharide (12 g.) was methylated three times with dimethyl sulphate and sodium hydroxide in the usual way. Two fractions were isolated, *viz.*, an insoluble fraction, purified by washing, drying in chloroform, and precipitating in light petroleum (4·5 g.) {OMe, 25·6%; $[\alpha]_D^{17°}$ −114° (in chloroform); uronic anhydride, nil}, and a soluble fraction (2 g.) [OMe, 15·1%; $[\alpha]_D^{17°}$ +78° (*c*, 3·692 in water); uronic anhydride, 14·7%].

Typical Hydrolysis of the Methylated Polysaccharide.—Fraction A (Table I) (30 g.) was boiled with methanolic hydrogen chloride (3%; 600 c.c.) till the rotation became constant ($[\alpha]_D^{15°}$ +55°, after 20 hours.) After neutralisation with silver carbonate and filtration, the solution was concentrated to give a non-reducing syrup (31·7 g.). Previous attempts had been made to fractionate a similar material entirely by high-vacuum distillation, but no clear-cut separation could be achieved.

Fractionation by Solvent Extraction.—The above syrup (31·7 g.) was first distilled in a Claisen flask, fitted with a vacuum-jacketed column, to give: (1) 11·511 g., b. p. 100—110°/0·08 mm., $n_D^{19°}$ 1·4411; (2) 3·310 g., b. p. 110—148°/0·04 mm., $n_D^{19°}$ 1·4517; and (3) 1·105 g., b. p. 148—155°/0·04 mm., $n_D^{19°}$ 1·4638. Fraction (2) was combined with the still-residue, dissolved in water (30 c.c.), and extracted for 9 hours with light petroleum (b. p. 38—40°) to give fraction (4), 3·714 g., $n_D^{17°}$ 1·4446. The residual aqueous solution was then extracted with chloroform to give (*a*) (½ hour) 1·344 g., $n_D^{17°}$ 1·4636, (*b*) (+1½ hours) 3·514 g., $n_D^{17°}$ 1·4690, (*c*) (+3 hours) 1·680 g., $n_D^{18°}$ 1·4727, (*d*) (+6 hours) 2·260 g., $n_D^{18°}$ 1·4747, (*e*) (+9 hours) 1·219 g., $n_D^{16°}$ 1·4777, and (*f*) (+15 hours) 1·059 g., $n_D^{18°}$ 1·4800. After treatment with charcoal, the residual aqueous solution was filtered hot and evaporated to a syrup (*g*), 2·547 g., $n_D^{18°}$ 1·4800. Fractions (3), (*a*), and (*b*) were combined, dissolved in water (50 c.c.), and extracted with light petroleum (b. p. 38—40°) for 96 hours, to give a syrup (*h*), 1·818 g., $n_D^{19°}$ 1·4550. The aqueous residue was evaporated to yield a syrup (*i*), 3·819 g., $n_D^{20°}$ 1·4702. Recovery was 29·627 g. (93%).

Hydrolysis and Fractionation of B.—Fraction B (Table I) (6·40 g.) was hydrolysed as above, the solution showing finally $[\alpha]_D^{15°}$ +58° after 28 hours. This yielded a non-reducing syrup (6·80 g.), which was dissolved in water (50 c.c.) and extracted continuously with light petroleum (b. p. 38—40°) to give fractions (*j*) (1½ hours), 1·526 g., $n_D^{22°}$ 1·4393, (*k*) (+2½ hours) 0·939 g., $n_D^{22°}$ 1·4398, and (*l*) (+12 hours) 0·854 g., $n_D^{22°}$ 1·4432. The aqueous residue was evaporated to 30 c.c. and extracted with chloroform to give fractions (*m*) (½ hour), 0·701 g., $n_D^{28°}$ 1·4595, (*n*) (+1½ hours) 0·610 g., $n_D^{20°}$ 1·4650, (*o*) (+5½ hours) 0·682 g., $n_D^{23°}$ 1·4713, (*p*) (+9 hours) 0·461 g., $n_D^{17°}$ 1·4713, and (*q*) (+12 hours) 0·252 g., $n_D^{16°}$ 1·4760. The aqueous solution was then evaporated to yield a syrup (*r*), 0·792 g., $n_D^{16°}$ 1·4866. Fractions (*n*), (*o*), and (*p*) were combined, dissolved in water (50 c.c.), and extracted with light petroleum for 10 hours to give fraction (*s*), 0·137 g., $n_D^{21°}$ 1·4520. The aqueous residue on evaporation yielded a syrup (*t*), 1·312 g., $n_D^{18°}$ 1·4724. Recovery was 6·513 g. (96%).

Examination of the Fully Methylated Fractions (1), (4), (j), (k), (l).—Preliminary attempts to separate these materials by chromatography on alumina were unsuccessful. Fraction (l) (11·3 g.) was hydrolysed by nitric acid (2%; 150 c.c.) at 100°. $[\alpha]_D^{15°}$ were +15° (zero time), +16° (1 hour), +9° (2 hours), +6° (3 hours), +4° (4 hours), and +3° (5 hours, constant). The solution was then neutralised with barium carbonate, filtered, and evaporated to a reducing syrup (9·7 g.). Crops of crystalline material, removed over a prolonged period (6·35 g.), showed m. p. 91°, alone or on admixture with an authentic specimen of trimethyl α-D-xylopyranose, and $[\alpha]_D^{15°}$ +50·8° (5 minutes), +31·1° (90 minutes), +30° (120 minutes, constant) (Found: OMe, 49·5. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The residual syrup (1·83 g.) had $[\alpha]_D^{15°}$ −15° (*c*, 1·392 in water) (equilibrium value). This syrup (1·55 g.) was dissolved in water (10 c.c.), bromine (2 c.c.) was added, and the mixture kept at room temperature until non-reducing (4 days). After the usual treatment with silver carbonate, followed by hydrogen sulphide, and lactonisation, a syrup resulted which was distilled, giving fractions (i), 0·52 g., b. p. 90—105°/0·10 mm., $n_D^{16°}$ 1·4510 (Found: OMe, 47·9. Calc. for $C_8H_{14}O_5$: OMe, 48·9%), and (ii) 0·35 g., b. p. 105—125°/0·10 mm., $n_D^{16°}$ 1·4590 (Found: OMe, 49·8%). Lactone fraction (i) showed $[\alpha]_D^{15°}$ −35° (5 minutes), −31° (20 hours),

−28° (50 hours), −23° (120 hours), −19° (170 hours), and −16° (310 hours, constant) (*c*, 0·577 in water). 11·5 Mg. on titration with 0·0254N-sodium hydroxide to phenolphthalein required 2·41 c.c. (Calc. for $C_8H_{14}O_5$: 2·39 c.c.), and on treatment with methanolic ammonia yielded crystalline 2 : 3 : 5-trimethyl L-arabonamide which on recrystallisation (ethyl acetate) had m. p. 137—138°, alone or in admixture with an authentic specimen, and $[\alpha]_D^{16°}$ +21° (*c*, 1·3 in water) (Found: C, 46·3; H, 8·2; N, 6·6; OMe, 44·6. Calc. for $C_8H_{17}O_5N$: C, 46·3; H, 8·3; N, 6·8; OMe, 44·9%). Lactone fraction (ii) showed $[\alpha]_D^{18°}$ −11° (5 minutes), −9° (20 hours), −5° (50 hours), and +2° (170 hours, constant) (*c*, 0·546 in water); 27·3 mg. required 6·15 c.c. of 0·0254N-sodium hydroxide (Calc. for $C_8H_{14}O_5$: 5·65 c.c.); it gave a syrupy amide, $[\alpha]_D^{16°}$ +35° (in water).

Paper-chromatographic investigations on the hydrolysed, fully methylated fractions showed the presence in each of trimethyl xylopyranose with a small amount of 2 : 4-dimethyl xylose.

Preparation of 2 : 3 : 4-Trimethyl Xylopyranose Anilide.—Crystalline trimethyl xylose (0·21 g.), freshly-distilled aniline (0·11 g.), and absolute ethanol (2 c.c.) were heated under reflux for 1½ hours. The alcohol was removed in a vacuum-desiccator till only *ca.* 0·5 c.c. of solution remained, and nucleated with a crystal of tetramethyl galactopyranose anilide. Crystallisation overnight yielded 2 : 3 : 4-*trimethyl xylose anilide*, obtained on recrystallisation from ether as colourless crystals, very similar in appearance to trimethyl xylose, having m. p. 98—100° and $[\alpha]_D^{19°}$ −84° (4 minutes), −75° (20 minutes), ±0° (195 minutes), +34° (345 minutes), and +47° (24 hours, constant) (*c*, 1·84 in ethanol). When the ethanol was allowed to evaporate at room temperature, the anilide was again obtained in crystalline form. Redissolution in ethanol gave a solution with a negative specific rotation, rapidly changing to a positive value as above (Found: C, 63·2; H, 7·6; N, 5·3; OMe, 35·0. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·2; OMe, 34·8%).

Examination of Dimethyl Methylpentoside Fractions.—Fraction (*h*) (1·810 g.) was dissolved in light petroleum–chloroform (2 : 1), and fractionation effected on a column of alumina (20 × 2 cm.), to give the following fractions (using the solvent as eluent): (1) 0·225 g., $n_D^{18°}$ 1·4450, (2) 0·405 g., $n_D^{18°}$ 1·4520, (3) 0·294 g., $n_D^{18°}$ 1·4565, (4) 0·166 g., $n_D^{18°}$ 1·4581, (5) 0·297 g., $n_D^{18°}$ 1·4581 (using chloroform as eluent), (6) 0·258 g., $n_D^{18°}$ 1·4581, and (7) 0·120 g., $n_D^{18°}$ 1·4575. Recovery was 1·765 g. (98%).

Fractions (3) to (7) were combined and hydrolysed with 2% nitric acid to yield a reducing syrup (0·91 g.), which crystallised completely to give 2 : 4-dimethyl β-D-xylopyranose, which, on recrystallisation (acetone–light petroleum), had m. p. 108°, unchanged on admixture with an authentic specimen. Treatment with ethanolic aniline gave crystalline 2 : 4-dimethyl xylose anilide, m. p. 166—168°, not depressed on admixture with an authentic specimen.

Lactone Formation.—Oxidation of the free sugar (0·30 g.) with bromine in water yielded the syrupy 2 : 4-dimethyl xylopyranolactone (0·20 g.), $n_D^{20°}$ 1·4698, $[\alpha]_D^{19°}$ +25° (in chloroform), and $[\alpha]_D^{19°}$ +9° (½ hour), +10° (1 hour), +11° (3 hours), +23° (23 hours), +29° (50 hours), and +30° (100 hours, constant) (in water). The lactone (12·8 mg.) required 3·48 c.c. of 0·0202N-sodium hydroxide for complete neutralisation (Calc. for $C_7H_{12}O_5$: 3·61 c.c.), and yielded syrupy 2 : 4-dimethyl xylonamide, which gave a negative Weerman test.

Periodic Acid Oxidation of the Free Sugar.—This was carried out according to the method of Reeves (*loc. cit.*). No formaldehyde was detected, whilst a similar estimation on D-xylose (0·0177 g.) yielded formaldehyde, identified as the dimedon complex (0·0309 g.), m. p. 188—190°.

The above findings were confirmed when it was shown, by means of the paper chromatogram that fractions (*h*), (*m*), and (*s*) consisted almost entirely of 2 : 4-dimethyl methylxylosides, with traces of trimethyl and monomethyl methylxylosides.

Examination of the Monomethyl Methylpentoside Fractions.—Fraction (*s*) on complete methylation and hydrolysis gave trimethyl α-D-xylopyranose, m. p. and mixed m. p. 87°, as the only product.

Hydrolysis. Fraction (*c*) (1·65 g.) was hydrolysed with 2% nitric acid (50 c.c.) at 100°. $[\alpha]_D^{20°}$ were +74° (initial value), +45° (1 hour), +34° (2 hours), +29° (3 hours), +25° (4 hours), +23° (5 hours), and +21° (6 hours, constant). The reducing syrup (1·32 g.) obtained failed to crystallise.

Anilide formation. The syrup (0·23 g.) was converted into a crystalline anilide. Recrystallisation from ethyl acetate gave 3-*methyl xylose anilide* as white needles, m. p. 138°, unchanged on admixture with an authentic specimen, $[\alpha]_D^{18°}$ +77° (*c*, 0·8 in ethyl acetate) (Found: C, 60·0; H, 7·2; N, 5·9; OMe, 12·6. $C_{13}H_{17}O_4N$ requires C, 60·2; H, 7·2; N, 5·9; OMe, 12·9%).

Fractions (*d*), (*e*), (*i*), (*q*), and (*t*) were also hydrolysed as above and each yielded an identical anilide.

The anilide (0·12 g.) was heated with N-sulphuric acid (10 c.c.) at 100° for 3 hours. After neutralisation with barium carbonate, filtration, and washing with ether, the solution was concentrated to a syrup which did not crystallise. Periodic acid oxidation of the syrup (0·067 g.) yielded formaldehyde, identified as the dimedon complex (0·051 g.), m. p. and mixed m. p. 187°.

Periodic Acid Oxidation Experiments on the Free Sugar.—The hydrolysed fraction (*c*) was submitted to periodic acid oxidation by Reeves's method, and an average formaldehyde yield of 27% was obtained in three experiments. Similar results were obtained from fractions (*d*), (*e*), (*i*), (*q*), and (*t*).

Methylfuranoside Formation.—Fraction (*c*) (0·2435 g.) was dissolved in methanol. The calculated amount of concentrated methanolic hydrogen chloride was added, and the solution was diluted to 25 c.c. with methanol. The total acid content was 0·5%. The solution showed $[\alpha]_D^{16°}$ +34° (initial) +32° (4 hours), +27° (6 hours), +26° (8 hours), +22° (24 hours), and +21° (48 hours, constant) (*c*, 0·974). A sample of the pyranoside (prepared independently) in the same solvent showed $[\alpha]_D^{16°}$ +69° (*c*, 0·984). Portions (2 × 1 c.c.) of the solution were withdrawn at intervals and examined for the presence of furanosides (Levene, Raymond, and Dillon, *J. Biol. Chem.*, 1932, **95**, 699), with results given in Table II.

Osazone Formation.—Hydrolysed fraction (*d*) (0·1 g.) was converted into the osazone. After 1 hour at 95° the solution was cooled, and the osazone separated as yellow needles. Recrystallisation from aqueous ethanol yielded 3-*methyl xylosazone*, m. p. 148—150° (Found: OMe, 7·1. $C_{18}H_{22}O_3N_4$ requires OMe, 9·1%).

TABLE II.

Time (hr.).	Free sugar, %.	Furanoside, %.	Pyranoside, %.
3	54·5	21·3	24·2
7½	34·9	28·0	37·1
24	27·3	35·6	36·1
48	30·3	33·9	35·8
72	26·3	27·1	46·6

Lactone Formation.—The free sugar (*c*) was converted into the lactone as usual and distilled. The distillate partly crystallised (69%).

Investigation of the Crystalline Lactone Fraction.—This was shown to consist of 3-*methyl xylopyranolactone* and on recrystallisation from ethyl acetate–light petroleum had m. p. 93—94°, unchanged on admixture with a synthetic specimen, and $[\alpha]_D^{15°}$ +76° (5 minutes), +73° (1½ hours), +69° (24 hours), +62° (144 hours), +50° (359 hours), +42° (652 hours), and +41° (820 hours, constant) (*c*, 0·74 in water). 14·8 Mg. required 1·75 c.c. of 0·05165N-sodium hydroxide for neutralisation (Calc. for $C_6H_{10}O_5$: 1·77 c.c.) (Found: C, 44·0; H, 6·2; OMe, 17·2. $C_6H_{10}O_5$ requires C, 44·4; H, 6·2; OMe, 19·1%). A sample of the lactone prepared from synthetic 3-methyl xylopyranose (Levene and Raymond, *J. Biol. Chem.*, 1933, **102**, 331) had m. p. 94—95° and $[\alpha]_D^{17°}$ +72° (5 minutes), +65° (20 hours), +60° (100 hours), +50° (290 hours), +44° (405 hours), and +41° (620 hours, constant) (in water). The crystalline lactone gave a syrupy *amide* (Found: OMe, 18·0. $C_6H_{13}O_5N$ requires OMe, 17·3%). The amide (0·05 g.) yielded hydrazodicarbonamide (0·011 g.), m. p. and mixed m. p. 256° (Weerman reaction).

Periodic Acid Oxidation.—The lactone (20 mg.) was converted into the sodium salt of the acid by heating with N-sodium hydroxide (0·5 c.c.) at 50° for 15 minutes. Treatment with periodic acid (Reeves, *loc. cit.*) gave formaldehyde as the dimedon complex in 75% yield.

Investigation of the Syrupy Lactone Fraction.—This had $n_D^{17°}$ 1·4851 (Found: OMe, 17·4. Calc. for $C_6H_{10}O_5$: OMe, 19·1%) and was obtained by extraction of the tile used in separating the crystalline component. Evaporation of the extracts yielded a syrup which distilled at 170°/0·02 mm. The colourless distillate had $[\alpha]_D^{17°}$ +46° (*c*, 1·448 in water), unchanged after 100 hours. 14·5 Mg. required 3·00 c.c. of 0·0207N-sodium hydroxide for neutralisation (Calc. for $C_6H_{10}O_5$: 4·32 c.c.). On conversion into the amide a syrup was obtained which gave a negative Weerman test.

On oxidising the sodium salt of the acid from the syrupy lactone with periodic acid as above, formaldehyde was again obtained, identified by the port-wine colour obtained with acid–phenylhydrazine–ferricyanide.

Anilide Formation and Fractional Crystallisation.—Syrup (*i*) was hydrolysed with 2% nitric acid, and the product (2·798 g.) converted into the anilide. The ethanol was removed by stages in a vacuum-desiccator to yield various crops of crystals. Crop I (0·015 g.) had m. p. 146—148°. Crop II (0·422 g.) on recrystallisation from ethyl acetate had m. p. 147—149°, unchanged on admixture with crop I (Found: C, 60·4; H, 7·6; N, 6·3; OMe, 23·1. Calc. for $C_{13}H_{19}O_4N$: C, 61·6; H, 7·5; N, 5·5; OMe, 24·5%). Crop III (0·280 g.) and crop IV (0·065 g.) were 3-methyl xylose anilide, (III) m. p. and mixed m. p. 137°, and (IV) m. p. and mixed m. p. with crop III, 140° [Found: OMe, (III) 13·5, (IV) 13·0%]. Crop V (0·850 g.) was impure 3-methyl xylose anilide, m. p. 128—130° not depressed on admixture with an authentic specimen (Found: OMe, 13·6%). Crop VI was obtained as a syrup on the removal of the remaining ethanol. Crops I and II were combined and decomposed with sulphuric acid to yield 2 : 4-dimethyl xylose identified on the paper chromatogram. Crop VI was decomposed with sulphuric acid to yield a reducing syrup.

Nitric Acid Oxidation of the Syrup from Crop VI.—The syrup (0·4 g.) in concentrated nitric acid (5 c.c.) was heated at 50° for 2 hours. The temperature was raised slowly and kept at 90° for a further 4 hours, whereafter the nitric acid was removed by repeated distillation with water, and the water removed by distillation with methanol. The solution was then evaporated to a syrup which was esterified with boiling 3% methanolic hydrogen chloride. The product was distilled at 145—170°/0·05 mm. The clear yellow distillate (0·08 g.) had $n_D^{13°}$ 1·4588 (Found: OMe, 39·9. Calc. for $C_8H_{14}O_7$: OMe, 41·9%), $[\alpha]_D^{18°}$ +39° (*c*, 0·72 in methanol). No crystalline amide could be obtained.

Paper Chromatogram Studies.—Examination of hydrolysed fractions (*c*), (*d*), (*e*), (*i*), (*q*), and (*t*) by means of the paper chromatogram showed that the only sugars present in each case were 3- and 2-methyl xylose, with traces of 2 : 4-dimethyl xylose and xylose.

Examination of the Residual Fractions.—Fraction (*f*), $n_D^{18°}$ 1·4800 (Found: OMe, 31·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), with 2% nitric acid gave a reducing syrup, $n_D^{14°}$ 1·4886. This, on periodic acid oxidation, yielded formaldehyde (47%), identified as the dimedon complex, and with alcoholic aniline yielded 3-methyl xylose anilide (20%), m. p. 136°, unchanged on admixture with an authentic specimen.

Fraction (*g*), $n_D^{18°}$ 1·4800 (CO_2Me, nil), gave, by hydrolysis, a reducing syrup which yielded crystalline D-xylose, m. p. 138° not depressed on admixture with an authentic specimen, $[\alpha]_D^{17°}$ +80° (2 minutes), +33° (40 minutes), and +17·5° (24 hours, constant) (*c*, 0·511 in water) (Found: OMe, nil).

Fraction (*r*), $n_D^{16°}$ 1·4866 (Found: OMe, 21·6. Calc. for $C_6H_{12}O_5$: OMe, 18·9%), when kept for a long time, deposited some crystalline material which was separated by trituration with ethyl acetate as white needles, m. p. 145° (micro) not depressed on admixture with a sample of β-methylxyloside. Hydrolysis of (*r*) gave a reducing syrup which yielded dibenzylidene xylose dimethyl acetal (Breddy and Jones, *loc. cit.*).

Demethylation Studies.—3-Methyl xylose (0·5 g., OMe, 18·2%) was boiled with methanolic hydrogen chloride (3%, 25 c.c.) for 23 hours, to yield a non-reducing syrup which was hydrolysed with 2% nitric acid, to give a reducing syrup (0·4 g.; OMe, 18·6%) which crystallised almost completely. Examination by means of the paper chromatogram showed that *ca.* 5% demethylation had taken place [Found: xylose, 0·7% (starting product); 6·0% (end product)]. A sample of 2 : 3 : 4-trimethyl xylose, on the same treatment, showed no appreciable demethylation.

Examination of the Polysaccharide regenerated from PI *Acetate.*—Acetate (*b*) (2 g.) was deacetylated

by shaking it with sodium hydroxide (N /10; 250 c.c.) at 15° for 24 hours. The solution was neutralised with glacial acetic acid and evaporatedto *ca.* 50 c.c. and the polysaccharide was precipitated by pouring into an excess of ethanol.

Hydrolysis.—The material (0·20 g.) was hydrolysed with 3% oxalic acid solution (25 c.c.) at 100°. $[\alpha]_D^{17°}$ were +7·5° (1 hour), +20° (2 hours), and +32·5° (4 hours, constant). The solution was neutralised with barium carbonate, filtered, concentrated to *ca.* 5 c.c., and poured into 100 c.c. of ethanol. No appreciable precipitate was obtained. Evaporation gave a syrup which (paper chromatogram) contained xylose and arabinose only, rhamnose being absent.

Formic Acid from regenerated PI.—The polysaccharide was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427), and the formic acid liberated was determined by titration with 0·01N-sodium hydroxide with the following results, expressed in mols. of formic acid/g.: 0·00034 (1 day); 0·00054 (3 days); 0·00111 (4 days); 0·00186 (7 days); 0·00227 (10 days, constant).

Periodate Uptake of regenerated PI.—PI (*ca.* 0·4 g.) was dissolved in water (50 c.c.), and sodium metaperiodate (*ca.* 2·14 g.) added. At intervals, 5-c.c. portions were withdrawn, and the periodate determined by the arsenite method, with the following results (expressed in mols. of periodate consumed per g.): 0·0076 (½ hour); 0·0087 (1 day); 0·0090 (2 days).

Study of Barium Salt X *from* PI.—*X* had $[\alpha]_D^{18°}$ +78° (in water) and Ba content 14·5%. *X* (24·2 g.) was further hydrolysed with 5% oxalic acid solution (400 c.c.) for 12 hours at 100°. This yielded a barium salt *Y* (13·5 g.) and a syrup *T* (6·5 g.). *T*, on investigation by the paper chromatogram was shown to contain only xylose and arabinose. The arabinose, estimated as the diphenylhydrazone, constituted 9% of the syrup, equivalent to *ca.* 1·4% of the original mucilage. *Y* ($[\alpha]_D^{18°}$ +82·8°; Ba, 17·3%) (10 g.) was hydrolysed with 5% sulphuric acid at 100° for 6 hours, neutralised, filtered, and treated as usual to give a barium salt *Z* (8·0 g.) and a reducing syrup (0·77 g.) which (paper chromatogram) consisted mainly of rhamnose, with traces of xylose and arabinose.

Z had $[\alpha]_D^{17°}$ +70° (*c*, 2·226 in water) (Found: Ba, 17·1; uronic anhydride, 37·3. Calc. for $C_{18}H_{18}O_{11}Ba_{\frac{1}{2}}$: Ba, 16·8; uronic anhydride, 43·2%). *Z* (2 g.) was oxidised by the method of Heidelberger and Goebel (*J. Biol. Chem.*, 1927, **74**, 616) to mucic acid (0·5 g.), m. p. 214° (decomp.), mixed m. p. with an authentic specimen, 215° (decomp.). *Z* (0·24 g.) was hydrolysed by 9% sulphuric acid (11 c.c.) at 100° for 18 hours, the solution showing finally $[\alpha]_D^{17°}$ +8·4°. The solution was then neutralised with barium carbonate and filtered, the filtrate concentrated to 5 c.c. and precipitated with ethanol, and the solvent removed to leave a reducing syrup. Examination on the paper chromatogram showed the presence of rhamnose with traces of xylose and arabinose.

Methylation of the Barium Salt Z.—*Z* (4·0 g.) was dissolved in water (70 c.c.), methyl sulphate (70 c.c.) added, and 30% sodium hydroxide solution (140 c.c.) run in during 4 hours, the mixture being stirred continuously (room temperature). After being kept overnight, the mixture was non-reducing. Solid sodium hydroxide (65 g.) was added, and methyl sulphate (110 c.c.) run in with stirring during 7 hours. The solution was again kept overnight, and was then heated at 100° for ½ hour, cooled, neutralised with concentrated sulphuric acid, and extracted with 6 × 300-c.c. portions of chloroform. Evaporation of the extracts yielded a syrup which was dissolved in water (50 c.c.) and remethylated with sodium hydroxide (150 c.c.) and methyl sulphate (75 c.c.) as above. After being kept overnight, the solution was worked up and extracted as before. The extracts were dried (Na_2SO_4), decanted, and concentrated to a syrup which was methylated four times with the Purdie reagents and twice with diazomethane. Distillation over barium carbonate at 170—190°/0·06 mm. yielded a yellow distillate (1·2 g.), $n_D^{18°}$ 1·4680 (Found: OMe, 48·7. Calc. for $C_{19}H_{34}O_{11}$: OMe, 49·5%).

The syrup (1·10 g.) was hydrolysed with aqueous hydrochloric acid (7%; 50 c.c.) at 100°; $[\alpha]_D^{17°}$ were +107° (zero time), +108° (1 hour), +96° (3 hours), +88° (5 hours), +81° (7 hours), +75° (9 hours), and +71° (12 hours, constant). The solution was cooled, neutralised with silver carbonate, and treated with hydrogen sulphide and barium carbonate. After evaporation, the residue was exhaustively extracted with anhydrous ether, leaving a white barium salt (0·6 g.). The ethereal extracts were combined and evaporated to yield a syrup (0·22 g.), $n_D^{18°}$ 1·4665, which, on drying at 40°/0·01 mm., crystallised completely. Recrystallisation from ether yielded 3 : 4-dimethyl α-L-rhamnose, m. p. 91—92° (Found; OMe, 30·1. Calc. for $C_8H_{16}O_5$: OMe, 32·3%), $[\alpha]_D^{17°}$ +14° (12 minutes), +19° (35 minutes, constant) (*c*, 0·688 in water).

The crystalline sugar was converted *via* the lactone into the amide, which gave a positive Weerman test.

The above barium salt (0·6 g.) was oxidised to methyl 2 : 3 : 4-trimethyl mucate (0·3 g.) (Gill, Hirst, and Jones, *J.*, 1939, 1469), m. p. 101·5°, alone or mixed with an authentic specimen, $[\alpha]_D^{17°}$ +29·0 (*c*, 0·715 in water) (Found: C, 46·9; H, 6·8; OMe, 53·4. Calc. for $C_{11}H_{20}O_8$: C, 47·1; H, 7·2; OMe, 55·4%).

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346. *Studies on Seed Mucilages. Part IV. The Seed Mucilage of* Plantago lanceolata.

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Methylated rib grass-seed mucilage prepared from the acetate (Part I, *J.*, 1940, 1501) gave, on methanolysis, trimethyl methyl-D-xylopyranosides (37%), tetramethyl methylgalactopyranosides (2·4%), 2 : 4-dimethyl methyl-D-xylosides (16%), 2 : 3-dimethyl methyl-D-xylosides (16%), 2 : 4 : 6-trimethyl methyl-D-galactosides (1·2%), 2-methyl methyl-D-xylosides (8%), 3-methyl methyl-D-xylosides (*ca.* 5%), and methyl-D-xylosides (7%).

The presence of 3 : 4-dimethyl xylose derivatives previously claimed (Part I, *loc. cit.*) could not be substantiated.

The aldobiuronic acid present in the portion of the mucilage resistant to hydrolysis appeared to be a mixture; the acidic component was identified as D-galacturonic acid.

In a preliminary examination of the mucilaginous polysaccharide from the seeds of the rib grass, *Plantago lanceolata* (Mullan and Percival, Part I, *J.*, 1940, 1501), among the products of hydrolysis of the methylated mucilage, 3 : 4-dimethyl xylose was thought to be present in a highly branched structure. James and Smith (*J.*, 1945, 744), however, isolated from methylated gum tragacanth a dimethyl xylose which gave a crystalline lactone of identical melting point with that reported in Part I, but showing $[\alpha]_D^{20°}$ −56° changing to −27° instead of $[\alpha]_D^{18°}$ +41° changing to +31° (in water). Evidence was presented which showed that the dimethyl xylonolactone isolated from the gum (James and Smith, *loc. cit.*) was probably 3 : 4-dimethyl D-xylonolactone. In order to clarify the position and to examine in more detail the constitution of rib grass-seed mucilage using improved methods of separating mixtures of methylated glycosides, the present investigation was undertaken.

The methoxyl content of the methylated mucilage obtained as in Part I (*loc. cit.*) could not be increased by using the thallium method. Fractional precipitation from chloroform solution by light petroleum gave two fractions *A* and *B*, and viscosity measurements indicated that the average molecular size of *A* was approximately twice that of *B*. Methanolysis of *A* was followed by distillation, to remove most of the fully methylated methylglycosides, the remainder being fractionated by extraction with light petroleum in a liquid extractor (Brown and Jones, *J.*, 1947, 1344), followed by chloroform. A fraction, insoluble in chloroform, remained. By these methods 2 : 3 : 4-trimethyl methyl-D-xylosides (37%) (identified as the crystalline trimethyl α-D-xylopyranose, the corresponding anilide, and by an examination of the lactone) and tetramethyl methyl-D-galactopyranosides (2·4%) (identified as the crystalline anilide) were separated. A search for fully methylated arabinose derivatives proved abortive.

After purification of the fractions extracted with light petroleum by adsorption on alumina (Jones, *J.*, 1944, 333) the following products were isolated: 2 : 4-dimethyl methyl-D-xylosides (16·1%), identified as the crystalline β-sugar and its anilide (Barker, Hirst, and Jones, *J.*, 1946, 783) and by the facts (*a*) that no formaldehyde was produced on the periodate oxidation of the free sugar (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476), proving substitution on $C_{(4)}$, and (*b*) that the derived amide gave a negative Weerman test, proving substitution on $C_{(2)}$; and 2 : 3-dimethyl methyl-D-xylosides (15·5%), identified as the crystalline amide (Hampton, Haworth, and Hirst, *J.*, 1929, 1739), the presence of a substituent on $C_{(3)}$ being confirmed by a negative Weerman test, and the absence of methoxyl residues on $C_{(4)}$ and $C_{(5)}$ by the formation of formaldehyde in almost theoretical yield (97%) on oxidation of the amide with periodate.

Early fractions extracted by chloroform were purified by chromatography on alumina and contained 2 : 4 : 6-trimethyl methyl-D-galactosides (1·2%), identified as the crystalline anilide (Hirst and Jones, *J.*, 1939, 1482), as well as the dimethyl methylxylosides already mentioned. A later chloroform fraction contained 2-methyl methyl-D-xylosides (8·4%), identified as the crystalline β-sugar (Robertson and Speedie, *J.*, 1934, 824), lactone, amide, and anilide, together with 3-methyl methylxylosides (*ca.* 5·0%). The evidence for the assignment of the methyl group to $C_{(3)}$ included the isolation of a monomethyl xylosazone, a positive Weerman test on the corresponding amide, the properties of the derived lactone, and the production of formaldehyde on oxidation with periodate (cf. Bell, *J.*, 1948, 992).

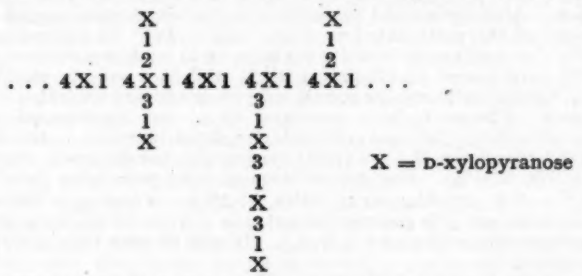
From the chloroform-insoluble residue crystalline β-methyl-D-xyloside was isolated. Methylxylosides (6·8%) were the main constituents of the residue, being identified as crystalline D-xylose, as the phenylosazone and by strip chromatography.

The following building units are present, therefore, in the portion of the mucilage isolated as the methylated derivative:

$$X1;\ 3X1;\ \overset{4}{X}1;\ \overset{4}{\underset{2}{X}}1;\ 3\overset{4}{X}1;\ 3\overset{4}{\underset{2}{X}}1;\ G1;\ 3G1$$

X = D-xylopyranose
G = D-galactopyranose

If the repeating unit is assumed to have one terminal D-galactopyranose residue, the inclusion of more than forty sugar residues would be necessary to explain the experimental results. If one neglects the galactose residues for the present and considers only xylose units, a structure such as that given below, of which there are many variants, could represent the general pattern.



The proportions of methylated methylxylosides which would be obtained on the methanolysis of a methylated polysaccharide constructed in the above fashion would be trimethyl 39% (37), 2 : 3-dimethyl and 2 : 4-dimethyl 18·2% (16), 2-methyl 8·4% (8·4), and 3-methyl methylxyloside 8·4% (5·3), and methylxylosides 7·8% (6·3); the experimental figures are given in parentheses, and have been corrected for demethylation found to occur during methanolysis (see Part III).

It will be noted that no 3 : 4-dimethyl xylose derivatives were recognised in the present series of experiments. Although the evidence that 3 : 4-dimethyl xylose was the principal constituent of the dimethyl pentose fraction appeared to be strong at the time (Part I, *loc. cit.*), it is now apparent that the dimethyl pentose fraction then examined was a complex mixture. The lactone thought to be 3 : 4-dimethyl D-xylonolactone, m. p. 67°, has now been found not to depress the melting point of 2-methyl D-xylonolactone (m. p. 66—68°). The constants quoted by James and Smith (*loc. cit.*) for 3 : 4-dimethyl xylonolactone are, therefore, inconsistent with the earlier views of one of us, and the claim that 3 : 4-dimethyl xylose is one of the principal products of the hydrolysis of methylated rib-grass-seed mucilage cannot be upheld. It should be pointed out, however, that, although the positive Weerman test (Part I, *loc. cit.*) might perhaps be explained by the presence of 3-methyl xylose in the mixture, the isolation of an optically active hydroxydimethoxyglutaric acid and D(−)-dimethoxysuccinamide remains unexplained.

No methylated derivatives of arabinose, rhamnose, or galacturonic acid were identified among the products of methanolysis. It is considered likely that the acidic nature of the polysaccharide as extracted from the seeds is due to an associated polyuronide which is eliminated on acetylation and methylation (cf. Part III, preceding paper), for the acetate was found to contain 7% of uronic anhydride and the methylated mucilage some 2·5%, falling to zero after four methylations.

A preliminary investigation of the barium salts obtained on hydrolysis of the mucilage with oxalic acid and neutralisation with barium carbonate was undertaken. The acidic portion was identified as D-galacturonic acid, but the original barium salt was certainly not a simple aldobiuronate. By hydrolysis using various conditions L-arabinose was obtained, and L-rhamnose, D-xylose, and D-galactose were also detected. A product approximating in composition to a barium aldobiuronate was isolated eventually and subjected to methylation and hydrolysis. The dimethyl ester of 2 : 3 : 4-trimethyl mucate was obtained on oxidation and esterification, but no conclusion has been reached as to the accompanying methylated pentose or methyl pentose residues.

Experimental.

All distillations were carried out under diminished pressure, and the temperatures quoted are bath-temperatures. The concentration of rotation solutions is 1% unless otherwise stated.

Preparation of the Mucilage.—The acid mucilage was extracted from the rib grass seeds as described in Part I (*loc. cit.*) (Found: uronic anhydride, 8·0%; equiv. by titration, 1025).

Autohydrolysis of the Mucilage.—The mucilage (0·5 g.) was heated with water (25 c.c.) at 95—100° for 20 hours, barium carbonate was added, the solution was filtered, concentrated to 10 c.c. at 40°, and poured

into methanol (300 c.c.) with stirring and the precipitate removed at the centrifuge. The alcoholic solution and washings were evaporated to dryness under reduced pressure, giving a syrup (0·09 g.) which was shown by strip chromatography to contain xylose and arabinose.

Oxidation with Periodate.—(*a*) The mucilage (0·553 g.) was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427) and the formic acid liberated was determined by titration with 0·0103N-sodium hydroxide. A correction was applied for the acidity of the mucilage. The results expressed in mols. of $H{\cdot}CO_2H$ per g. were 0·00018 (½ hour), 0·00055 (18 hours), 0·0016 (66 hours), 0·0022 (163 hours), and 0·0020 (211 hours). (*b*) The mucilage (0·451 g.) was dissolved in water (100 c.c.) containing sodium metaperiodate (4·233 g.). The periodate remaining was determined at intervals on 5-c.c. portions by the arsenite method [Found (mols. of $NaIO_4$ per g.) : 0·0020 (10 minutes), 0·0026 (70 minutes), 0·0029 (21½ hours), 0·0038 (141 hours), and 0·0042 (168 hours, constant); the uptake was initially very rapid].

Methylation.—Acetylation and subsequent methylation were carried out as described in Part I (*loc. cit.*). A sample of the methylated product, $[\alpha]_D^{15°}$ −105° (in chloroform) (OMe, 35·0%), on a further methylation by the thallium method did not increase in methoxyl content (34·4%). The uronic anhydride content of the methylated mucilage was 2·5%, and in a sample methylated four times with sodium hydroxide and methyl sulphate the uronic anhydride content was nil.

Fractionation.—The methylated mucilage (55 g.) was fractionated into two portions *A* and *B* by dissolving it in chloroform (700 c.c.) and adding light petroleum (b. p. 40—60°) slowly with stirring. After addition of light petroleum (3 l.), a stable precipitate was obtained, fraction *A* (34 g.), $[\alpha]_D^{17°}$ −103° (in chloroform) (OMe, 33·4%). Further addition of light petroleum (total, 4 l.) gave a second fraction, *B* (21 g.), $[\alpha]_D^{17°}$ −111° (in chloroform) (OMe, 34·2%). *A* had η_{sp}/c' 125 and *B* η_{sp}/c' 69·5 (where η_{sp} is the specific viscosity and c' is the concentration in g.-mols. of methylated anhydroxylose residues per l., assuming the repeating unit to be $C_5H_8O_4$). It will be seen that the apparent molecular size of *A* is almost twice that of *B*.

Hydrolysis of the Methylated Mucilage.—Three series of preliminary experiments (on 10-g. samples of the methylated polysaccharide) were carried out to determine the best conditions for separation : by distillation, by a combination of distillation and chromatographic adsorption on alumina, and by solvent extraction. In all cases the proportion of trimethyl methylxylosides obtained was similar to that arrived at in the experiments described below. Tetramethyl galactopyranose, 2 : 4 : 6-trimethyl galactose, and 2 : 4-dimethyl xylose were also identified. It was found that the dimethyl methylpentoside fraction amounted to about 30% of the methylated methylglycosides obtained on hydrolysis and appeared to consist exclusively of dimethyl methylxylosides since complete methylation and hydrolysis gave trimethylxylopyranose in good yield.

Fraction *A* (31·0 g.) was then hydrolysed with 3% methanolic hydrogen chloride (600 c.c.) for 22 hours. The mixture of glycosides obtained was distilled to remove most of the fully methylated portion (*a*) (9·08 g.), b. p. 105—130°/0·06 mm., $n_D^{18°}$ 1·4414. The glycosides remaining were dissolved in water (30 c.c.), barium carbonate (1 g.) was added, and the aqueous solution extracted with light petroleum (200 c.c., b. p. 38—40°) for 4 hours in a liquid extractor. The solvent was removed to give fraction (*b*) (4·24 g.), $n_D^{18°}$ 1·4490. Fractions (*a*) and (*b*) were combined and redistilled to give (*c*) (8·56 g.), b. p. 83—97°/0·01 mm., $n_D^{17°}$ 1·4413, (*d*) (1·58 g.), b. p. 97—110°/0·01 mm., $n_D^{19°}$ 1·4420, and (*e*) (1·37 g.) (undistilled residue), $n_D^{17°}$ 1·4542 (OMe, 50·1%).

Extraction was continued with light petroleum, giving (*f*) (3·03 g.), $n_D^{15°}$ 1·4560 (OMe, 47·3%) (24 hours), and (*g*) (7·65 g.), $n_D^{18°}$ 1·4595 (OMe, 47·0%) (94 hours). Extraction was continued with chloroform to remove the less fully methylated glycosides, and yielded (*h*) (2·48 g.), $n_D^{18°}$ 1·4690 (OMe, 37·5%) (2 hours), and (*i*) (4·68 g.), $n_D^{19°}$ 1·4746 (OMe, 29·0%) (24 hours). Evaporation of the remaining solution gave a brown glass (*j*) (2·70 g.). Recovery was 98%.

Examination of the Distillation Fractions.—Fractions (*c*) and (*d*) were combined (10·14 g.) and hydrolysed with 2% nitric acid (150 c.c.) for 5 hours at 100°. $[\alpha]_D^{17°}$ were +18·8° (initial), +24·5° (1 hour), +18·2° (2 hours), and +15·0° (5 hours). The reducing syrup obtained crystallised almost completely in 12 hours, but left a syrupy portion (0·30 g.), which was passed through an alumina column (28 × 1·8 cm.) using a mixture of methanol and chloroform (1 : 5) for development. The resulting syrup (0·17 g.) had $[\alpha]_D^{17°}$ +3·7° (in water), and $n_D^{18°}$ 1·4560, and was converted into the lactone (0·13 g.), b. p. 100—110°/0·01 mm., $n_D^{17°}$ 1·4585, which crystallised partly on standing, the crystals having m. p. 47—48°, unchanged on admixture with 2 : 3 : 4-trimethyl D-xylonolactone (m. p. 53°). The remaining syrupy lactone (0·068 g.) had $[\alpha]_D^{18°}$ ±0° (3 hours), +9·5° (40 hours), +13° (64 hours). This rotational change is characteristic of trimethyl D-xylopyranolactone and it appeared that no fully methylated arabofuranose derivative was present in this fraction. The crystalline sugar (8·0 g.), on recrystallisation from dry ether, had m. p. 88—90° unchanged on admixture with 2 : 3 : 4-trimethyl α-D-xylose, and $[\alpha]_D^{21°}$ +54° (initial), +35° (15 minutes), +27° (30 minutes), +23·5° (45 minutes), and +20° (1 hour, constant) (*c*, 1·2 in water). The sugar (0·80 g.) was oxidised to the lactone, giving on distillation 0·65 g., b. p. 110—125°/0·04 mm., which crystallised immediately. Recrystallised from dry ether gave needles, m. p. 53° [Found : C, 50·6; H, 7·4; OMe, 51·1. Calc. for $C_8H_{14}O_5$: C, 50·5; H, 7·4; OMe, 48·9%], $[\alpha]_D^{18°}$ +1·3° (initial), +4° (10½ hours), +10·5° (47½ hours), +16·5° (95½ hours), and +19° (120 hours, constant) (*c*, 1·6 in water). The anilide (0·16 g.), prepared from the crystalline 2 : 3 : 4-trimethyl xylose (0·48 g.), had m. p. 100—101° (Found : N, 5·3; OMe, 35·9. Calc. for $C_{14}H_{21}O_4N$: N, 5·2; OMe, 34·8%).

Fraction (*e*) (1·37 g.), dissolved in a mixture (25 c.c.) of light petroleum (b. p. 40—60°) and chloroform (4 : 1), was passed through an alumina column (25 × 3·5 cm.). Development was commenced using the original solvent and continued with a 2 : 1 mixture and finally with chloroform. Two main groups of fractions were collected : (i) $n_D^{18°}$ 1·4480—1·4489 (1·0 g.), and (ii) $n_D^{17°}$ 1·4540—1·4560 (0·3 g.). These were hydrolysed and, on conversion into the anilide, crystallised. The crystals (0·70 g.), removed on porous tile, had m. p. 192—193°, unchanged when mixed with an authentic sample of 2 : 3 : 4 : 6-tetramethyl galactose anilide (m. p. 192°), and $[\alpha]_D^{19°}$ −75° (*c*, 0·5 in acetone).

Examination of the Light-petroleum Fractions.—Fraction (*f*) (3·03 g.) was purified by an alumina column (30 × 1·8 cm.). Development was effected with a mixture of light petroleum and chloroform

(2 : 1) and then chloroform. One main group of fractions was collected, having $n_D^{19°}$ 1·4552—1·4565 (2·1 g.) and hydrolysed to a reducing syrup (1·9 g.) which crystallised partly. The crude product, removed on porous tile, crystallised from dry acetone by the addition of light petroleum (b. p. 80—100°) as needles (0·2 g.), m. p. 108—110°. The syrup (*g*) (7·65 g.), recovered from the tile by extraction with acetone, was purified by passage through alumina (20 × 3·5 cm.), and development effected by light petroleum–chloroform (1 : 1) and finally chloroform. Two main groups of fractions were collected: (i) $n_D^{19°}$ 1·4570—1·4582 (3·9 g.), and (ii) $n_D^{20°}$ 1·4552—1·4567 (1·5 g.). Fraction (i) was hydrolysed; the resulting reducing syrup (3·5 g.) crystallised partly; the crystals, recovered and recrystallised (1·1 g.) as described above, had m. p. 108—110°, not depressed on admixture with an authentic specimen of 2 : 4-dimethyl xylose (m. p. 108°).

Characterisation of 2 : 4-*dimethyl xylose.* The crystalline sugar (m. p. 108—110°) had $[\alpha]_D^{19°}$ +4° (17 minutes), +16° (67 minutes), +21° (2 hours), and +23° (3 hours constant) (*c*, 2·3 in water) (Found: C, 46·8; H, 8·0; OMe 33·8. Calc. for $C_7H_{14}O_5$: C, 47·1; H, 7·9; OMe, 34·8%). A portion of the crystalline sugar (0·13 g.) was oxidised to the lactone (0·07 g.), b. p. 130—135°/0·01 mm. $n_D^{14°}$ 1·4800 (last drop), $[\alpha]_D^{15°}$ −5° (initial), −1·5° (2 hours), +5° (5½ hours), +17° (24 hours), and +26° (72 hours, constant) (*c*, 0·6 in water). This rotational change is characteristic of a δ-xylonolactone. The syrupy amide (0·05 g.) was prepared from the lactone (0·05 g.) and gave a negative Weerman test, indicating the presence of a methoxyl group on $C_{(3)}$. The sugar (0·034 g.) was oxidised with periodate, but no formaldehyde was obtained (dimedon complex), indicating that $C_{(4)}$ was occupied by a methoxyl group.

The syrup recovered from the tile by acetone extraction, after removal of the crystalline 2 : 4-dimethyl xylose, was examined by strip chromatography; development of the chromatogram with ammoniacal silver nitrate produced spots corresponding to 2 : 4- (R_G value 0·66) and 2 : 3-dimethyl xylose (R_G value 0·74). The syrupy mixture (0·78 g.) was converted into the lactone (0·56 g.), b. p. 135—140°/0·05 mm., $n_D^{18°}$ 1·4750, $[\alpha]_D^{15°}$ +65·5° (initial), +50° (17½ hours), +55° (43 hours), +54·5° (113 hours), +51·5° (210 hours), +49° (600 hours) (*c*, 1·4 in water), a portion of which was converted into the amide; in the Weerman test this gave a small precipitate of hydrazodicarbonamide, corresponding to some 14% of a sugar not substituted on $C_{(3)}$, perhaps due to the presence of small amounts of 3 : 4-dimethyl xylose or 3-methyl xylose; a control experiment was run simultaneously on gluconamide.

Characterisation of 2 : 3-*dimethyl xylose.* Fraction *g* (ii) was hydrolysed, but the reducing syrup (1·4 g.) obtained did not crystallise. The derived lactone (0·91 g.) had b. p. 125—135°/0·05 mm., $n_D^{17°}$ 1·4665. $[\alpha]_D^{15°}$ +63° (initial), +63° (6 hours), +65° (24 hours), +64° (96 hours), +61° (192 hours), +57° (264 hours) and +54° (576 hours) (*c*, 0·8 in water), the rotational change being characteristic of a γ-lactone. The lactone (0·29 g.) was converted into the crystalline amide (0·15 g.), m. p. 132—133°, not depressed on admixture with an authentic sample of 2 : 3-dimethyl xylonamide (m. p. 133°), which gave a negative "Weerman" test indicating the presence of a methoxyl group on $C_{(2)}$ (Found: C, 43·5; H, 7·9; N, 7·0; OMe, 31·3. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%). A portion of the amide (0·027 g.) was oxidised with periodate; a quantitative yield of the formaldehyde–dimedon complex (0·040 g.) confirmed the absence of methoxyl groups on $C_{(4)}$ and $C_{(5)}$.

Investigation of the Chloroform Fractions.—Fraction (*h*) was distilled, giving 1·45 g., b. p. 110—120°/0·1 mm., $n_D^{22°}$ 1·4650 (OMe, 41·6%), and a waxy residue (0·9 g.) (not carbohydrate in nature). The distillate was purified by passage through alumina (28 × 1·8 cm.), and development effected by light petroleum–chloroform (2 : 1), then by chloroform, and finally by chloroform–methanol (4 : 1). One main group of fractions ($n_D^{18°}$ 1·4570—1·4580; 0·53 g.) was collected and then hydrolysed. The anilide of the resulting reducing syrup (0·48 g.) was fractionally crystallised; the first crop (0·07 g.) had m. p. 172°, unchanged on admixture with authentic 2 : 4 : 6-trimethyl galactose anilide (m. p. 172°), and $[\alpha]_D^{15°}$ −97° (initial), −87° (½ hour), −26° (5½ hours), +3° (11 hours), and +31° (23 hours, constant) (*c*, 0·3 in acetone); the second crop (0·1 g.) had m. p. 143° which was lowered by recrystallisation; the third crop (0·1 g.), m. p. 109—118°, gave a crystalline amide, m. p. 128—131°, unchanged on admixture with authentic 2 : 3-dimethyl xylonolactone (m. p. 133°), by way of the sugar and lactone. Fraction (*i*) (4·67 g.) was purified on an alumina column (17 × 3·5 cm.); light petroleum–chloroform (1 : 2), followed by chloroform and finally a chloroform–methanol mixture (2 : 1), were used for development. One main group of fractions, $n_D^{18°}$ 1·4700—1·4735 (3·4 g.), was collected and then hydrolysed to give a reducing syrup (3·1 g.) which was dissolved in the minimum quantity of ethanol; slow removal of the solvent gave crystals (1·1 g.), m. p. 135—137°.

Characterisation of 2-*methyl xylose.* The crystals, m. p. 135—137°, had $[\alpha]_D^{16°}$ −23° (initial), −1° (15 minutes), +20° (35 minutes), +34° (2 hours), and +35° (24 hours, constant) (*c*, 3·5 in water) (Found: C, 44·3; H, 7·7; OMe, 19·9. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). Periodate oxidation and quantitative determination of the formaldehyde–dimedon complex indicated that 86% of the sugar was not substituted on $C_{(4)}$ and $C_{(5)}$. The crystalline sugar (0·1 g.) was converted into the osazone (0·04 g.) (OMe, 1·0%), m. p. 144°, mixed m. p. with an authentic sample of xylose phenylosazone (m. p. 156—158°) 154—155°. The sugar (0·35 g.) was oxidised to the lactone (0·23 g.), b. p. 155—160°/0·01 mm., $n_D^{14°}$ 1·4832. Crystallisation occurred after several days; m. p. 66—68°, unchanged on admixture with the supposed 3 : 4-dimethyl xylonolactone (m. p. 67°) (Part I, *loc. cit.*); $[\alpha]_D^{17°}$ +101° (initial), +98° (5 hours), +93° (71 hours), +81° (288 hours), and +74° (504 hours). The lactone (0·06 g.) gave a crystalline amide (0·04 g.), m. p. 96—98°, $[\alpha]_D^{18°}$ +52·5° (*c*, 2·0 in water) (Found: C, 40·0; H, 6·9, N, 8·3; OMe, 16·7. Calc. for $C_6H_{13}O_5N$: C, 40·2; H, 7·3; N, 7·8; OMe, 17·3%). The amide gave a negative Weerman test indicating the presence of a substituent on $C_{(3)}$. The anilide prepared from the sugar had m. p. 125—126°, $[\alpha]_D^{18°}$ +214° (*c*, 2·2 in ethyl acetate) (Found: C, 60·0; H, 7·1; N, 6·1; OMe, 12·8. Calc. for $C_{13}H_{17}O_4N$: C, 60·3; H, 7·1; N, 5·9; OMe, 12·9%).

Characterisation of 3-*methyl xylose.* After complete removal of the alcohol and crystalline sugar from fraction (i), a syrup was left which on periodate oxidation gave a 65% yield of formaldehyde (cf. Bell, *J.*, 1948, 992, for the oxidation of 3-methyl glucose). Examination of this syrupy fraction by strip chromatography showed, on development with ammoniacal silver nitrate, an elongated spot similar to that given by a mixture of 2- and 3-methyl xyloses. The osazone (0·10 g.), prepared from the syrup (0·18 g.),

was purified by passage through an alumina column (20 × 1·8 cm.), benzene–methanol (49 : 1) being used for development. Two bands were obtained; the less strongly adsorbed yielded an osazone (0·02 g.), m. p. 166—168°, unchanged on admixture with an authentic sample of 3-methyl xylose phenylosazone (m. p. 172°) (Found : OMe, 8·7. Calc. for $C_{18}H_{22}O_3N_4$: OMe, 9·1%). The syrup (0·30 g.) was converted into the lactone (0·11 g.), b. p. 160—170°/0·05 mm., $n_D^{18°}$ 1·4854, $[\alpha]_D^{17°}$ +67° (initial), +67° (5 hours), +63° (95 hours), +50° (287 hours), and +42° (528 hours, constant); the rotational change appeared to exclude the possibility of the presence of a δ-lactone such as would be given by 4-methyl xylose. The lactone was converted into the amide which gave hydrazodicarbonamide in the Weerman test, the yield, in comparison with gluconamide, indicating that 49% of the sugar was not substituted on $C_{(3)}$.

Isolation of β-Methylxyloside and D-*Xylose.*—A portion of the residue (*j*) (0·5 g.) was dissolved in the minimum quantity of ethanol and the solvent slowly removed. Crystals (0·1 g.), m. p. 154°, $[\alpha]_D$ −32° (*c*, 0·8 in water), were obtained. Hydrolysis and examination of the reducing syrup by strip chromatography showed only xylose to be present, confirming that the above crystalline product was β-methyl-D-xyloside. Hydrolysis of the remainder (2·2 g.) of the fraction and crystallisation from ethanol gave a crystalline sugar (1·2 g.), m. p. 141°, unchanged on admixture with authentic D-xylose (m. p. 141°). Examination, by strip chromatography, of the syrupy fraction remaining after removal of the solvent confirmed the presence of xylose. The development with ammoniacal silver nitrate also showed the presence, in this fraction, of small quantities of monomethyl xyloses, galactose, and an unidentified sugar (R_G value 0·25) which may be a monomethyl hexose.

Attempted Characterisation of the Barium Aldobiuronate.—(a) *Graded hydrolysis of the mucilage.* The acid mucilage (117 g.) was hydrolysed with 3% oxalic acid solution (2 l.) for 20 hours at 100°. The barium salt (10·3 g.) was isolated, as described in Part I (*loc. cit.*), as a pale yellow amorphous powder. This product (10·3 g.) was treated again with oxalic acid (200 c.c.; 3%) for 6 hours at 100°; the barium salt (4·0 g.) gave CO_2 7·3 and Ba 10·1%. The alcoholic solution of sugars (0·8 g.) obtained from the second hydrolysis was examined by strip chromatography, which showed the presence of xylose, rhamnose, and arabinose in approximately equal proportions, with a smaller quantity of galactose. The presence of L-arabinose was confirmed by the isolation of L-arabinose diphenylhydrazone (m. p. 204°) and L-rhamnose by the isolation of the L-rhamnose monohydrate (m. p. 90—94°) (0·03 g.). Hydrolysis was continued using 6% sulphuric acid (50 c.c.) for 6 hours at 100°; the barium salt (2·7 g.) obtained gave CO_2 9·4 and Ba 15·4%, whilst examination of the ethanolic solution of sugars (0·07 g.) by strip chromatography showed the presence of arabinose with traces of galactose and xylose.

(b) *Hydrolysis of the barium aldobiuronate.* The barium aldobiuronate (0·25 g.) was hydrolysed with 9·8% sulphuric acid (25 c.c.) for 25 hours at 100°. $[\alpha]_D^{17°}$ were +60° (initial), +54° (1 hour), +46° (5 hours), +24° (21½ hours) and +22° (25 hours). Samples were withdrawn, neutralised with barium carbonate, and run on a strip chromatogram against standards. A gradual increase in the intensity of the spot due to arabinose was observed from 1 to 25 hours, with an inverse change in intensity of the spot on the starting line due to the uronic and aldobiuronic acid. Traces of rhamnose and galactose were also present.

(c) *Oxidation with bromine and hydrobromic acid.* The barium aldobiuronate (0·5 g.) was dissolved in hydrobromic acid (6 c.c.; 6%) containing bromine (0·25 c.c.) and heated under reflux for 8 hours at 100°. During 14 days at 0° crystals separated, m. p. 208—209°, unchanged on admixture with an authentic sample of mucic acid (m. p. 213°). The filtrate yielded a small quantity of the benziminazole of L-arabonic acid; m. p. 231—233°; mixed m. p. with an authentic sample (m. p. 240°) 234—236°.

(d) *Methylation.* The barium aldobiuronate (1·8 g.) was methylated three times with sodium hydroxide and methyl sulphate, three times with silver oxide and methyl iodide, and once with diazomethane. Distillation gave fractions (i) (0·32 g.), b. p. 115—130°/0·05 mm., $n_D^{18°}$ 1·4503 (OMe, 54·1%), and (ii) (0·51 g.), b. p. 170—190°/0·05 mm., $n_D^{18°}$ 1·4687 (OMe, 49·1%). Fraction (ii) was hydrolysed with 7% hydrochloric acid (25 c.c.) for 10½ hours at 100°; $[\alpha]_D^{17°}$ were +121° (initial), +116° (1 hour), +95° (6 hours), and +82° (10½ hours). After the usual treatment with silver carbonate, hydrogen sulphide, and barium carbonate, a syrup was obtained which was exhaustively extracted with boiling ether, to give a syrup (*x*) (0·12 g.) and a residue (*y*) (0·40 g.) containing some inorganic material. (*x*) was examined by strip chromatography and on development with ammoniacal silver nitrate afforded two spots (R_G values 0·54 and 0·77, respectively). The mixed lactones (0·05 g.), prepared from the sugar, had b. p. 115—130°/0·01 mm., $n_D^{19°}$ 1·4635, and $[\alpha]_D^{18°}$ +20° (initial), +10° (8 hours), and +2° (constant value). They were apparently only δ-lactones. An amide (0·02 g.), prepared from the lactone (0·04 g.), had m. p. 147—148° (Found : C, 43·0; H, 7·85; N, 7·1; OMe, 28·2. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%). A mixed m. p. with an authentic sample of 3 : 4-dimethyl L-rhamnonamide (m. p. 144—145°) was 110—126°. The analytical figures suggested that the compound was a dimethyl pentonamide, but investigation of the unmethylated aldobiuronate suggested an arabinose structure. The dimethyl arabonolactones which could form δ-lactones are 2 : 3-, 2 : 4-, and 3 : 4-dimethyl arabinose. 2 : 3-Dimethyl arabonamide has m. p. 162°, and 2 : 4-dimethyl arabonamide m. p. 158°, neither figure being in agreement with the value found (147—148°). There remains the possibility that the 3 : 4-dimethyl arabopyranose linked through $C_{(3)}$ is involved in the structure of the methylated aldobiuronic acid.

The residue (*y*) was oxidised with bromine to the methylated mucic acid, esterified by boiling with methanolic hydrogen chloride (25 c.c.; 3%) for 23 hours, and neutralised by addition of silver carbonate; the filtrate was evaporated, giving a white residue which was exhaustively extracted with boiling ether. Concentration of these extracts gave a syrup (0·14 g.) which was distilled, having b. p. 120—135°/0·01 mm., $n_D^{19°}$ (1·4580 (OMe, 56·1%), and crystallising partly (m. p. 94—98°, unchanged on admixture with an authentic sample of the dimethyl ester of 2 : 3 : 4-trimethyl mucic acid).

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347. *Experiments on the Synthesis of Purine Nucleosides. Part XXII. The Synthesis of the α- and β-Forms of 9-Triacetyl-D-ribopyranosido-2-methylthioadenine and Further Studies on the Synthesis of 9-Glycofuranosidopurines.*

By G. W. Kenner, H. J. Rodda, and A. R. Todd.

In an endeavour to extend the Schiff-base route to 9-glycofuranosidopurines outlined in Part XVII (*J.*, 1948, 957) to the synthesis of adenosine, *5-benzoyl* 2 : 3 : 4-*triacetyl* D-*ribose* was condensed with 4 : 6-diamino-2-methylthiopyrimidine, and the product converted into 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* by a method similar to that used for the corresponding arabinoside in Part XVII (*loc. cit.*). Although it had been expected that this product would be a furanoside, it yielded on reduction, thioformylation, and sodium alkoxide cyclisation, followed by reacetylation, 9-*triacetyl*-β-D-*ribopyranosido*-2-*methylthioadenine*; the same glycoside was obtained by an analogous series of reactions from 6-*amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine* prepared by direct condensation of D-ribose and 4 : 6-diamino-2-methylthiopyrimidine. The intermediate *azo-glycosids* in this case was quite distinct from that obtained by the Schiff-base route. Both of these azo-glycosides, when reduced, thioformylated, and cyclised with potassium acetate in methyl cyanide yielded 9-*triacetyl*-α-D-*ribopyranosido*-2-*methylthioadenine*. The significance of these results is discussed, and a probable explanation suggested which also brings into line anomalous results obtained in a re-examination of the L-arabinoside series investigated in Part XVII (*loc. cit.*). The reason for the difficulties experienced with the Schiff-base route to furanosides employing a protecting benzoyl group is pointed out, and a solution of these difficulties proposed.

In Part XVII of this series (Kenner, Lythgoe, and Todd, *J.*, 1948, 957), we described the synthesis of 9-L-arabofuranosido-2-methylthioadenine by a method depending on the condensation of 4 : 6-diamino-2-methylthiopyrimidine with 5-benzoyl 2 : 3 : 4-triacetyl L-arabinose to a product believed to be a Schiff base. Removal of the acetyl groups from this material by partial hydrolysis yielded, by isomerisation, a furanosidamino-pyrimidine which was then used for synthesising the purine glycoside by the general procedure laid down in earlier papers of this series. The natural development of this Schiff-base route was to apply it to the synthesis of adenosine (9-β-D-ribofuranosidoadenine) by using D-ribose in place of L-arabinose and desulphurising the 9-D-ribofuranosido-2-methylthioadenine so obtained by means of Raney nickel, a method already applied in analogous cases (Part XI; Howard, Lythgoe, and Todd, *J.*, 1945, 556).

D-*Ribose diethyl thioacetal* was prepared by a route analogous to that employed for D-lyxose diethyl thioacetal by Wolfrom and Moody (*J. Amer. Chem. Soc.*, 1940, **62**, 3465), converted into its *5-benzoyl* derivative and thence into *5-benzoyl* 2 : 3 : 4-*triacetyl* D-*ribose diethyl thioacetal*. From the last compound the free *aldehydo*-sugar was obtained as a syrup which could not be crystallised; it was condensed with 4 : 6-diamino-2-methylthiopyrimidine, and the crude product used directly to prepare, by the method described in Part XVII (*loc. cit.*), a 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*. This azo-glycoside, which we expected to have a furanose structure, had $[\alpha]_D$ +320° (approx.). Zinc dust reduction, followed by treatment with dithioformic acid, yielded a thioformamido-compound which was cyclised with sodium methoxide in the usual manner. No crystalline product could be isolated directly from the cyclisation reaction, but addition of picric acid precipitated a *picrate* of 9-D-ribosido-2-methylthioadenine. After acetylation the picrate was decomposed by passing it in chloroform solution through a column of activated alumina. The crystalline glycoside so obtained had m. p. 230°, and from its analysis, positive Molisch reaction, ultra-violet absorption spectrum, and the fact that, after deacetylation, it consumed 3 moles of periodate, it was clearly 9-*triacetyl*-D-*ribopyranosido*-2-*methylthioadenine* and not the expected ribofuranoside.

For purposes of comparison, 9-triacetyl-D-ribopyranosido-2-methylthioadenine was synthesised by application of our general synthetic method to 6-*amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine*. The latter compound was first prepared in these laboratories some years ago by Dr. H. T. Howard; although crystalline and apparently homogeneous, the possibility that it may be a mixture of α- and β-isomers is not wholly excluded. The 6-amino-4-triacetyl-D-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine prepared from it in the usual manner was commonly obtained as an amorphous powder of $[\alpha]_D$ −100° and was used in this form for the next stage in the synthesis; a small fraction obtained crystalline on one occasion had m. p. 182—183°. This azo-glycoside, then, was clearly different from the supposedly

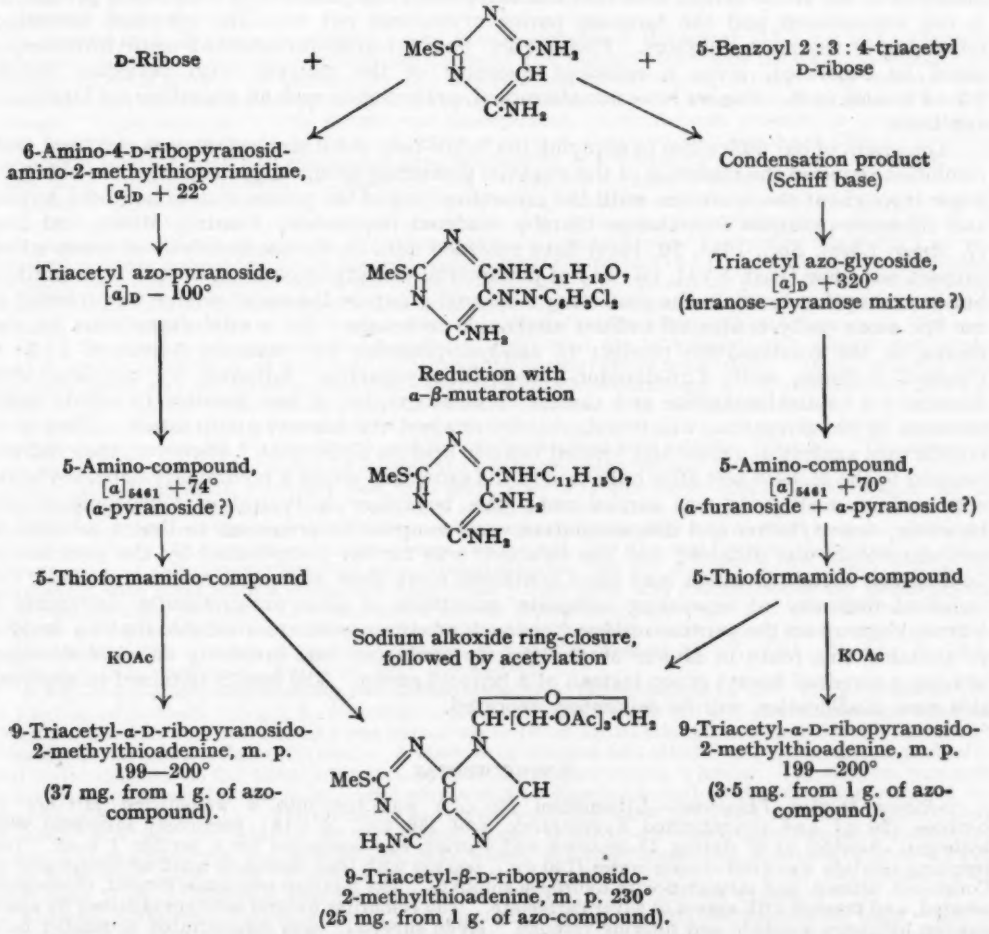
furanose azo-glycoside mentioned above, which had $[\alpha]_D$ +320°, but on reduction, thioformylation, and cyclisation with sodium methoxide, followed by acetylation, it gave a 9-triacetyl-D-ribopyranosido-2-methylthioadenine, m. p. 230°, identical with that described above, and gave it, moreover, in closely similar yield.

These results and other considerations led us to the view that α–β-isomerisation, accompanied by furanose–pyranose interconversion, might be taking place during the alkoxide-catalysed ring-closure. To obviate furanose–pyranose interconversion, it would be necessary to retain the protecting acetyl groups on the sugar residue during cyclisation, but the only method of which we were aware fulfilling these conditions gave large quantities of 6-glycosidaminopurines (cf. Kenner and Todd, Part XIII, *J.*, 1946, 852). Trial experiments, however, showed that potassium acetate in methyl cyanide formed a suitable medium giving, in some cases at least, good yields of 9-glycosidopurines; thus, 6-amino-4-triacetyl-D-xylosidamino-5-thioformamido-2-methylthiopyrimidine cyclised in this medium gave a 45% yield of 9-triacetyl-D-xylopyranosido-2-methylthioadenine, although cyclisation in potassium acetate–acetic acid gave mainly 6-triacetyl-D-xylosidamino-2-methylthiopurine.

The 5-thioformamido-compound, prepared from the, presumably, pyranose 6-amino-4-triacetyl-D-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine ($[\alpha]_D$ −100°), was therefore heated with potassium acetate in methyl cyanide. Even after chromatographic purification the product could not be directly crystallised, but it yielded a crystalline picrate, m. p. 130—132°, quite different from the picrate (m. p. 209—210°) of the 9-triacetyl-D-ribopyranosido-2-methylthioadenine (m. p. 230°) obtained previously by alkoxide cyclisation. Decomposition of the picrate yielded a crystalline purine glycoside, m. p. 199—200°. Analysis and periodate titration confirmed that this product was indeed a second 9-triacetyl-D-ribopyranosido-2-methylthioadenine. Its ultra-violet absorption spectrum was indistinguishable from that of the isomeric compound, but it had $[\alpha]_D$ +94°, whereas the isomer of m. p. 230° had $[\alpha]_D$ −34°. Clearly, the purine, m. p. 199—200°, is the α- and that of m. p. 230° is the β-D-*ribopyranoside*. The " A-value " calculated from the molecular rotations is 28,000, which is in reasonable agreement with the value 23,200 found in the D-xylopyranoside series (Howard, Kenner, Lythgoe, and Todd, Part XV, *J.*, 1946, 861). Repetition of these experiments showed that the yields of purine glycoside were reproducible and similar with the two methods of ring-closure, but the potassium acetate–methyl cyanide method always gave the α-glycoside, m. p. 199—200°, without any detectable quantity of the β-isomer, whereas sodium alkoxide gave invariably the β-glycoside. The general theory of the interconversion reactions of *N*-glycosides expounded in Part XIV of this series (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) provides a reasonable explanation of these phenomena. 5 : 6-Diamino-4-triacetyl-D-ribopyranosidamino-2-methylthiopyrimidine will undergo a rapid mutarotation to an equilibrium mixture consisting largely of the more stable αβ-isomer. Although no definite assessment can yet be made, our observation that this material in solution has an optical rotation of *ca.* + 74° is compatible with its being composed largely of the α-riboside, which would be expected to yield the α-thioformamido-derivative. On cyclisation with potassium acetate in a non-hydroxylic medium, this thioformamido-compound yields the purine α-riboside, m. p. 199—200°. On the other hand, in hot sodium alkoxide solution the acetyl groups are rapidly removed thereby changing the relative stabilities of the α- and β-isomers, and the conditions are extremely favourable for mutarotation, particularly when a proton is removed either from the 5-thioformamido- or from the 4-glycosidamino-group. The production of a purine β-riboside under these conditions is not surprising; all previous syntheses by this general route for purine nucleosides have yielded β-glycosides.

The supposedly furanose acetyl azo-riboside ($[\alpha]_D$ +320°) was now reduced and thioformylated and the product cyclised with potassium acetate in methyl cyanide. Here too the only product isolated was 9-triacetyl-α-D-ribopyranosido-2-methylthioadenine, but the yield was much lower, in our opinion significantly lower, than that from the authentic pyranose acetyl azo-riboside. These results could be explained on the assumption that the 5 : 6-diamino-4-triacetyl-D-ribosidamino-2-methylthiopyrimidine obtained by reducing the azo-compound ($[\alpha]_D$ +320°) is a mixture of α-furanoside and α-pyranoside, an assumption which would not conflict with the fact that its rotation in solution (+70°) does not differ widely from that of the authentic pyranose compound. Thioformylation of this material and cyclisation with potassium acetate in methyl cyanide would give a corresponding mixture of purine ribosides from which only the α-pyranoside can be isolated in crystalline form. Sodium methoxide, on the other hand, by removing the protecting acetyl groups would allow furanose–pyranose interconversion as well as α–β-mutarotation to proceed unchecked during cyclisation, so that

from either starting material identical yields of β-pyranoside would be expected. The results obtained in the ribose series, with our suggested explanation of them, are summarised in the accompanying scheme.



Although there is no proof of the validity of these hypotheses, their plausibility is enhanced by a re-examination of the L-arabinoside series studied in Part XVII (*loc. cit.*). There we had originally applied the sodium alkoxide cyclisation and obtained from the supposedly furanose acetyl azo-arabinoside a 9-L-arabinosido-2-methylthioadenine, which we believed to be a pure furanoside. The complex situation now uncovered in the D-ribose series made a re-examination of the arabinose derivatives imperative. Repetitions of the synthesis described in Part XVII (*loc. cit.*) gave a crystalline nucleoside, but as a rule this product consumed about 2·6 moles of periodate on titration and thus appeared to be a mixture of furanose and pyranose compounds. We were unable to obtain again a product with a periodate titration value as low as that described in Part XVII (*loc. cit.*). Mr. C. W. Taylor in this laboratory has found that when sodium ethoxide is used for cyclisation a similar material (periodate uptake 2·6 mols./mol.) is produced starting from *6-amino-4-triacetyl-L-arabinosidamino-5-(2′ : 5′-dichlorobenzeneneazo)-2-methylthiopyrimidine*, which, prepared as it was in normal fashion from the condensation product of L-arabinose and 4 : 6-diamino-2-methylthiopyrimidine, would be expected to be a pyranose compound. Recrystallisation of this apparently furanose–pyranose mixture of purine glycosides does not separate it into its components, and the periodate uptake remains unchanged. Chromatography of the triacetate, however, yields, with heavy loss, a product from which by deacetylation pure 9-L-*arabopyranosido-2-methylthioadenine* can be obtained. The same pyranoside is afforded by potassium acetate–methyl cyanide cyclisation of the pyranose

thioformamido-compound, but the supposedly furanose thioformamido-compound, prepared as described in Part XVII (*loc. cit.*), gives a purine arabinoside with a significantly lower periodate consumption (2·3—2·4 mols./mol.). These phenomena are fundamentally similar to those observed in the ribose series, save that the exceptional complication of α-glycoside production is not encountered and the furanose purine crystallises out with the pyranose instead of remaining in the mother-liquors. Presumably, pure 9-L-arabofuranosido-2-methylthioadenine could be separated, given a sufficient quantity of the material with periodate uptake 2·3—2·4 mols./mol.; this we have not attempted, preferring to seek an unambiguous furanoside synthesis.

The origin of our difficulties in applying the Schiff-base route successfully was clear and their resolution required the retention of the essential protecting group in position-5 of the *aldehydo*-sugar throughout the synthesis, until the glyoxaline ring of the purine system had been formed and furanose–pyranose interchange thereby rendered impossible. Gordon, Miller, and Day (*J. Amer. Chem. Soc.*, 1948, **70**, 1946) have recorded data on the ammonolysis of esters which support our view (Part XVII, *loc. cit.*) of preferential deacetylation as against debenzoylation, but in attempting to retain the protecting 5-benzoyl group on the sugar residue we preferred to use the more easily controlled sodium methoxide technique. By a mild deacylation by this means on the condensation product of *aldehydo*-pentoses, for example, 5-benzoyl 2 : 3 : 4-triacetyl D-ribose, with 4 : 6-diamino-2-methylthiopyrimidine, followed by coupling with diazotised 2 : 5-dichloroaniline and careful chromatography, it was possible to isolate small amounts of azo-glycosides, which undoubtedly retained the benzoyl group intact. They gave satisfactory analytical values and yielded benzoic acid on hydrolysis. Moreover, they did not respond to the Molisch test after reduction of the azo-group unless a preliminary debenzoylation with sodium methoxide was carried out; this behaviour is typical of 5-benzoylpentoses. However, deacetylation and debenzoylation were competitive processes, so that a mixture of azo-compounds was obtained and the situation was further complicated by the presence of unidentified materials which may have contained more than one sugar residue. Indeed, the technical difficulty of separating adequate quantities of pure azo-glycosides containing a 5-benzoyl group on the pentose residue from such mixtures were so formidable that we decided to abandon this route in favour of the strictly analogous one involving an *aldehydo*-sugar bearing a terminal benzyl group instead of a benzoyl group. The results obtained in applying this new modification will be separately reported.

Experimental.

D-*Ribose Diethyl Thioacetal.*—Ethanethiol (36 c.c.) was run into a well-stirred mixture of D-ribose (30 g.) and concentrated hydrochloric acid (36 c.c.; *d* 1·18; previously saturated with hydrogen chloride) at 0° during 15 minutes and stirring was continued for a further ½ hour. The resulting solution was diluted with water (150 c.c.), treated with lead carbonate until no longer acid to Congo-red, filtered, and saturated with hydrogen sulphide. The mixture was again filtered, thoroughly aërated, and treated with excess of silver carbonate. The colourless neutral solution obtained by again passing hydrogen sulphide and filtering through "Hyflo supercel" was concentrated to smaller bulk (150 c.c.), whereupon D-*ribose diethyl thioacetal* (25 g.) separated; a further quantity (6·5 g.) was obtained by concentrating the mother-liquors. Recrystallised from water or aqueous ethanol, it formed colourless needles, m. p. 82—83°, and had $[\alpha]_D^{13}$ −41·5° (*c*, 0·5 in water) (Found: C, 41·8; H, 7·7. $C_9H_{20}O_4S_2$ requires C, 42·2; H, 7·8%).

5-*Benzoyl* D-*Ribose Diethyl Thioacetal.*—Benzoyl chloride (10·4 c.c.) in pyridine (40 c.c.) was added dropwise during 1 hour to a well-stirred solution of D-ribose diethyl thioacetal (25·6 g.) in pyridine (60 c.c.), cooled in an ice–salt mixture. Stirring was continued for 4 hours, and the mixture kept at room temperature overnight, then poured into ice-water (300 c.c.), and stirred for 1 hour. The precipitated 5-*benzoyl* derivative was collected and recrystallised from benzene–light petroleum (b. p. 60—80°) (3 : 1). It formed colourless needles (19 g.), m. p. 100°, $[\alpha]_D^{14°}$ 0° (*c*, 0·16 in chloroform) (Found, in material dried at 65°: C, 53·2; H, 6·7. $C_{16}H_{24}O_5S_2$ requires C, 53·3; H, 6·7%). No formaldehyde could be detected after oxidising a specimen with periodic acid.

5-*Benzoyl* D-*Ribose Phenylhydrazone.*—5-Benzoyl D-ribose diethyl thioacetyl (1·8 g.), cadmium carbonate (4·1 g.), mercuric chloride (4·1 g.), and water (60 c.c.) were stirred vigorously for 30 minutes at room temperature and then for 1 hour at 80°, filtered, cooled, and saturated with hydrogen sulphide. The filtered solution was aërated, treated with excess of silver carbonate, and again filtered. On renewed saturation with hydrogen sulphide at 0°, filtration, aëration, and evaporation to dryness under reduced pressure, 5-benzoyl D-ribose was obtained as a syrup (0·98 g.). Treated with phenylhydrazine hydrochloride in presence of sodium acetate, this product gave 5-*benzoyl* D-*ribose phenylhydrazone* which crystallised from aqueous ethanol as yellow plates, m. p. 159° (Found: N, 8·5. $C_{18}H_{20}O_3N_2$ requires N, 8·1%).

5-*Benzoyl* 2 : 3 : 4-*Triacetyl* D-*Ribose Diethyl Thioacetal.*—Acetic anhydride (50 c.c.) was added dropwise during 1 hour to a stirred ice-cold solution of 5-benzoyl D-ribose diethyl thioacetal (18 g.) in pyridine (80 c.c.). The mixture was stirred for a further 3 hours, kept overnight at room temperature,

then poured into ice-water (800 c.c.), and extracted twice with chloroform. The combined chloroform extracts were washed with, successively, acetic acid (10%), water, sodium hydrogen carbonate, and water, dried, and evaporated under reduced pressure. The *triacetyl* compound crystallised when set aside and was washed with a little hexane and recrystallised from the same solvent, to give colourless needles, m. p. 47—48°, $[\alpha]_D^{15°}$ +14·5° (*c*, 0·15 in chloroform) (Found: C, 54·0; H, 6·2. $C_{22}H_{30}O_8S_2$ requires C, 54·3; H, 6·2%).

5-Benzoyl 2 : 3 : 4-*Triacetyl* D-*Ribose*.—The above acetylated thioacetal (15·5 g.) and cadmium carbonate (30 g.) were stirred in acetone (58 c.c.) and then diluted with water (20 c.c.). A solution of mercuric chloride (31·2 g.) in acetone (58 c.c.) was added gradually during 1 hour. The mixture was stirred at room temperature for 20 hours and then for ½ hour at 50—70° before being cooled and filtered through "Hyflo supercel." The filtrate was concentrated, under reduced pressure in presence of a little cadmium carbonate, to a thick oil which was then dissolved in chloroform (200 c.c.). The chloroform solution dried and evaporated gave the *product* as a colourless resin which distilled at 170° (bath temp.)/10^{-4} mm. (Found: C, 57·3; H, 5·3. $C_{18}H_{20}O_9$ requires C, 56·8; H, 5·3%).

*6-Amino-4-triacetyl-*D*-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* ($[\alpha]_D$ +320°), *prepared by the Schiff-base Route*.—A boiling solution of 4 : 6-diamino-2-methylthiopyrimidine (20·2 g.) and ammonium chloride (0·52 g.) in dry ethanol (480 c.c.) was added to the syrupy 5-benzoyl 2 : 3 : 4-triacetyl D-ribose prepared from the corresponding diethyl thioacetal (15·5 g.), and the resulting solution set aside for 2 days. Solvent was removed by evaporation, and the residue extracted four times with chloroform (200 c.c. in all); unchanged diamine (12 g.) remained undissolved. The combined chloroform extracts were evaporated and the residue was set aside for 2 days with methanolic ammonia (320 c.c. of 5N.); the solution was concentrated to 100 c.c. and boiled for 30 minutes with sodium methoxide (1·47 g. of sodium in 30 c.c. of methanol) to remove the benzoyl group completely. Acetic acid (3·5 c.c.) was then added and the whole poured into a neutral solution of diazotised 2 : 5-dichloroaniline (8·1 g.). After 15 minutes the yellow precipitate was collected, washed with water, and dried at 50° *in vacuo*.

The crude product (20 g.) so obtained was dissolved in pyridine (100 c.c.) and adsorbed on active alumina (500 g.; column, 8·5-cm. diam.), and the column washed with pyridine (500 c.c.). The adsorbed azo-compound was next acetylated by stirring the adsorbate with pyridine and acetic anhydride (75 c.c.) in the cold, setting aside overnight, and then stirring for a further hour with addition of fresh acetic anhydride (25 c.c.). Ethanol (75 c.c.) was now added and the cooled mixture stirred for 1 hour. The alumina adsorbate was then extracted five times with equal portions (200 c.c.) of ethyl acetate containing 5% of pyridine. The combined extracts were evaporated, and the residue was redissolved in ethyl acetate (50 c.c.) and chromatographed on activated alumina (200 g.; column, 5-cm. diam.). Washing the column with ethyl acetate eluted the main broad yellow band, and evaporation of the eluate gave a dark reddish resin (6·2 g.). The resin could not be crystallised but, when this was dissolved in hot ethanol and allowed to cool, the *acetyl azo-glycoside* separated as an amorphous orange powder, $[\alpha]_D^{18°}$ +320° ±30° (*c*, 0·3 in chloroform) (Found, in material dried at 80°: C, 45·1; H, 4·6; N, 14·2. $C_{22}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%).

*6-Amino-4-*D*-ribopyranosidamino-2-methylthiopyrimidine* (Experiment by Dr. H. T. Howard).—A solution of D-ribose (10 g.), 4 : 6-diamino-2-methylthiopyrimidine (20 g.), and ammonium chloride (0·6 g.) in absolute ethanol (120 c.c.) was heated under reflux for 30 minutes in a flask fitted with an 18″ Fenske column and a reflux-ratio head. A mixture of benzene and ethanol (1 : 1) was added gradually and water removed as the ternary mixture by slow distillation during 4 hours. The solution was now poured on activated alumina (1 kg.), and excess of 4 : 6-diamino-2-methylthiopyrimidine removed by washing with alcohol (2 l.). The glycoside was eluted with cold water (5 l.), and the eluate concentrated to small bulk and set aside at 0° for several days. *6-Amino-4-*D*-ribopyranosidamino-2-methylthiopyrimidine* separated and was recrystallised from water; it formed rosettes of colourless needles (6·2 g.), m. p. 138—140°, $[\alpha]_D^{18°}$ +22° (*c*, 0·25 in water) (Found: C, 39·2; H, 6·0; N, 17·5; loss on drying at 110°, 5·5. $C_{10}H_{16}O_4N_4S,H_2O$ requires C, 39·2; H, 5·9; N, 17·6; loss on drying, 5·8%).

*6-Amino-4-triacetyl-*D*-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* ($[\alpha]_D$ −100°).—A neutral solution of diazotised 2 : 5-dichloroaniline (3·2 g.) was added to 6-amino-4-D-ribopyranosidamino-2-methylthiopyrimidine (4·1 g.) in water (10 c.c.), and after 30 minutes the yellow precipitate was collected, washed with water, and dried *in vacuo*. The product was dissolved in pyridine (36 c.c.) containing acetic anhydride (9 c.c.), and the solution set aside overnight. Ethanol (9 c.c.) was then added and after 1 hour the solution was evaporated under reduced pressure, and the residual resin dissolved in ethyl acetate (75 c.c.) and adsorbed on a column of activated alumina (180 g.; 5-cm. diam.). Washing the column with ethyl acetate eluted most of the coloured material which formed a broad yellow band, and evaporation of the eluate yielded a yellow resin. When this resin was dissolved in hot ethanol and the solution allowed to cool, the *acetyl azo-glycoside* separated as a yellow powder (4·5 g.), $[\alpha]_D^{17°}$ −100° ±8° (*c*, 0·13 in chloroform) (Found, in material dried at 80°: C, 45·1; H, 4·2; N, 13·8. $C_{22}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%). When an ethyl acetate solution of this material evaporated slowly, the compound separated on one occasion as long needles, m. p. 182—183°, $[\alpha]_D^{15°}$ −320° ±20° (*c*, 0·13 in chloroform) (Found, in material dried at 100°: C, 45·6; H, 4·3; N, 13·6%).

Rotation of Crude 5 : 6-*Diamino-4-triacetyl-*D*-ribosidamino-2-methylthiopyrimidines prepared from the Above Azo-glycosides*.—Parallel reductions with zinc dust and acetic acid were carried out on each of the above azo-glycosides (0·5 g.). The 5-amino-glycosides obtained were resins; their optical rotations were determined in chloroform (80 c.c.). The product obtained from the azo-glycoside ($[\alpha]_D$ +320°) had $[\alpha]_{5461}^{20°}$ +70°, whilst that from the azo-glycoside ($[\alpha]_D$ −100°) had $[\alpha]_{5461}^{20°}$ +74°.

*9-Triacetyl-β-*D*-ribopyranosido-2-methylthioadenine*.—(a) *From triacetyl azo-riboside* ($[\alpha]_D$ +320°). Zinc dust (16 g.) was stirred vigorously with a solution of the azo-compound (2 g.) in boiling ethyl acetate (60 c.c.), and a mixture of acetic acid (8 c.c.) and ethyl acetate (80 c.c.) was gradually added during 1 hour. The solution was decanted and the residue extracted thrice with hot ethyl acetate. The combined solution and extracts were evaporated under reduced pressure (nitrogen), and the resinous amino-compound obtained was dissolved in methanol (100 c.c.) and cooled to 0°. A freshly prepared solution of dithioformic acid (1·4 g. of sodium dithioformate in 10 c.c. of methanol, treated at 0° with

0·4 g. of acetic acid in 10 c.c. of methanol) was added and the mixture kept at 0° for 1 hour and then set aside at room temperature overnight. A fresh portion of dithioformic acid (from 0·7 g. of sodium salt) was added, and the mixture heated under reflux for 1 hour and evaporated to dryness under reduced pressure. The residue was extracted four times with ethyl acetate (100 c.c. in all), and the solution concentrated to 20 c.c., poured on a column of neutral alumina (70 g.; 3·5-cm. diam.), washed with ethyl acetate (260 c.c.), and finally eluted with pyridine (130 c.c.). Evaporation of the eluate gave 6-amino-5-thioformamido-4-triacetyl-D-ribosidamino-2-methylthiopyrimidine as a pale yellow resin (676 mg.) which did not crystallise and was used directly for further work.

The resinous thioformamido-compound (400 mg.) was dissolved in ethanol (20 c.c.), sodium methoxide (95 mg.) added, and the mixture heated under reflux for 4 hours and then evaporated to dryness under reduced pressure. The residue was dissolved in water (7 c.c.) and neutralised with dilute hydrochloric acid, and the flocculent precipitate spun off and washed with water (4 × 3 c.c.). The combined filtrate and washings were mixed with picric acid (7 c.c. of a saturated ethanolic solution) and set aside overnight at room temperature. The yellow precipitate was extracted with boiling water (50 c.c.), and the extract concentrated to half bulk and set aside. The crystalline picrate so obtained (100 mg.) had m. p. 160—165° (decomp.). It was dissolved in pyridine (9 c.c.), and acetic anhydride (1·5 c.c.) added. After 16 hours ethanol (1·5 c.c.) was added, and the mixture set aside for 1 hour and then evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (15 c.c.) and poured on a column of activated alumina (10 g.; 1-cm. diam.) which retained the picric acid, the acetylated purine glycoside being washed through the column with chloroform (100 c.c.). Evaporation of the chloroform washings, followed by recrystallisation of the residue from alcohol, gave 9-*triacetyl-β-*D-*ribopyranosido-2-methylthioadenine* as colourless prisms (20 mg.), m. p. 230° (Found, in material dried at 100°: C, 46·7; H, 5·2; N, 15·6. $C_{17}H_{21}O_7N_5S$ requires C, 46·5; H, 4·8; N, 15·9%). The compound was insoluble in alkali, had $[\alpha]_D^{17°}$ −35° ±3° (*c*, 0·36 in pyridine) and in ethanol showed absorption maxima at 2345 A. (ε, 24,300) and 2760 A. (ε, 15,700). A small amount (8·2 mg.) was deacetylated with sodium methoxide (2 mg.) in methanol (10 c.c.). After removal of methanol the residue was dissolved in water, made slightly acid, and oxidised with 0·25M-sodium metaperiodate (1 c.c.); in 40 hours the consumption of periodate was complete at 3·0 mols./mol. The riboside yielded with alcoholic picric acid a picrate, m. p. 209—210° (decomp.).

(b) *From triacetyl azo-riboside* ($[\alpha]_D$ −100°).—The triacetyl azo-compound (1·5 g.) was converted into the corresponding 5-thioformamido-compound and cyclised with sodium methoxide as described above. On being worked up in the same manner, 9-triacetyl-β-D-ribopyranosido-2-methylthioadenine (17 mg.), m. p. 230°, was obtained. A mixture of this product with that obtained by method (*a*) showed no depression in m. p.

*Cyclisation of 6-Amino-5-thioformamido-4-triacetyl-*D*-xylopyranosidamino-2-methylthiopyrimidine with Potassium Acetate in Methyl Cyanide.*—A solution of the thioformamido-compound (160 mg.; *cf.* Part XI, *loc. cit.*) in dry methyl cyanide (10 c.c.) was heated under reflux for 14 hours with freshly fused potassium acetate (98 mg.; 3 mols.). The residue remaining after evaporation of the solvent was extracted with chloroform (5 c.c.). Removal of chloroform, followed by replacement with ethanol (2 c.c.), yielded 9-triacetyl-D-xylopyranosido-2-methylthioadenine (47 mg.); recrystallised from alcohol it had m. p. 192°, undepressed by an authentic specimen (m. p. 192—193°). The original alcoholic mother-liquor, treated with sodium ethoxide, yielded a small additional amount of product as 9-D-xylopyranosido-2-methylthioadenine (12 mg.).

9-*Triacetyl-α-*D-*ribopyranosido-2-methylthioadenine.*—(a) *From 6-amino-4-triacetyl-*D*-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* ($[\alpha]_D$ +320°). The acetyl azo-compound (2·0 g.) was converted into the resinous 5-thioformamido-glycoside as described for the preparation of 9-triacetyl-β-D-ribopyranosido-2-methylthioadenine (above). The thioformamido-compound (676 mg.), fused potassium acetate (412 mg.), and dry methyl cyanide (50 c.c.) were heated under reflux for 14 hours with exclusion of moisture, and the solution was evaporated to dryness under reduced pressure. The residue was thrice extracted with chloroform (40 c.c. in all), and the extracts were evaporated, yielding a yellow resin (486 mg.) which did not crystallise. It was accordingly re-dissolved in chloroform (10 c.c.) and adsorbed on neutral alumina (50 g.; column, 3·5-cm. diam.). The main pale yellow band was washed through with chloroform (200 c.c.) and chloroform–ethanol (9 : 1; 150 c.c.), the eluate evaporated, the residue (210 mg.) dissolved in warm ethanol (3 c.c.), and saturated ethanolic picric acid (3 c.c.) added. The picrate which separated was washed with cold alcohol (3 × 1 c.c.) and recrystallised from water; the final product (70 mg.) had m. p. 128—130°. The picrate was dissolved in chloroform (10 c.c.) and decomposed by adsorption on alumina in the usual manner, yielding a pale brownish resin (32 mg.) which was dissolved in a minimum of warm benzene, *n*-hexane being then added to incipient turbidity. When the mixture was kept for several days in the ice-chest, 9-*triacetyl-α-*D-*ribopyranosido-2-methylthioadenine* separated as clumps of colourless micro-crystals (7 mg.) and was recrystallised from the same solvent mixture. The purified material was insoluble in alkali and had m. p. 199—200° and $[\alpha]_D^{18°}$ +94° ±5° (*c*, 0·148 in pyridine) (Found, in material dried at 100°: N, 15·7. $C_{17}H_{21}O_7N_5S$ requires N, 15·9%). In ethanol the substance showed absorption maxima at 2345 A. (ε, 23,800) and 2755 A. (ε, 15,800). A portion of the glycoside (6·217 mg.) was deacetylated with sodium methoxide (2 mg.) in the usual way, and the product oxidised with 0·25M-periodate (1 c.c.); after 40 hours the consumption of periodate corresponded to 3·0 mols./mol., confirming the pyranose nature of the synthetic product. The picrate prepared by treatment by alcoholic picric acid had m. p. 130—132°.

(b) *From 6-amino-4-triacetyl-*D*-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* ($[\alpha]_D$ −100°). The azo-compound (0·76 g.) was reduced, thioformylated, and cyclised with potassium acetate in methyl cyanide as described in (*a*) above. 9-Triacetyl-α-D-ribopyranosido-2-methylthioadenine (28 mg.) was obtained, having m. p. 199—200°, undepressed in admixture with the material obtained by method (*a*).

*6-Amino-4-triacetyl-*L*-arabinosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine ("Pyranose" Azo-compound).*—L-Arabinose (5 g.), 4 : 6-diamino-2-methylthiopyrimidine (10 g.), and ammonium chloride (0·3 g.) were heated under reflux in absolute ethanol (80 c.c.) for 1 hour in a flask

fitted with an 18" Fenske column with reflux-ratio head. A mixture of benzene and ethanol (1 : 1; 200 c.c.) was added in portions, and water removed as the ternary mixture by slow distillation. After 8-hours' heating the solution was set aside overnight and worked up in the usual way by adsorption on alumina and elution of the condensation product with water. Concentration of the eluate yielded the glycoside as a resin which could not be crystallised and was used directly for the next step. Dissolved in water (80 c.c.) it was added to a neutral solution of diazotised 2 : 5-dichloroaniline (5·4 g.), and after 1 hour the precipitated azo-compound (9·9 g.) was collected, washed with water, and dried. The crude azo-glycoside was acetylated with acetic anhydride (15 c.c.) in pyridine (45 c.c.) by setting the mixture aside overnight. Ethanol (20 c.c.) was added, and after 2 hours the solution was evaporated under reduced pressure and the residue dissolved in ethyl acetate and chromatographed on neutral alumina in the usual manner. The *triacetyl azo-arabinoside* was eluted from the column with ethyl acetate, and evaporation of the eluate yielded it as an amorphous or micro-crystalline powder (2·7 g.). On dissolution of it in ethanol containing a little ethyl acetate and slow evaporation of the solution, this material yielded small orange prisms, m. p. 213—214°, $[\alpha]_D^{20°}$ −209° (*c*, 0·42 in chloroform) (Found, in material dried at 140°: C, 44·7; H, 4·2; N, 14·3. $C_{23}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%).

6-*Amino*-4-L-*arabinosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Methanolic sodium methoxide (50 mg. of sodium in 2 c.c. of methanol) was added to a solution of the above acetyl azo-arabinoside (0·5 g.) in chloroform (3 c.c.), and the mixture kept overnight at room temperature. The deacetylated *azo-arabinoside* which separated was recrystallised from pyridine–alcohol; it formed flat orange-coloured prisms, m. p. 233—234° (Found, in material dried at 140°: N, 17·9. $C_{16}H_{18}O_4N_6SCl_2$ requires N, 18·2%).

9-L-*Arabopyranosido*-2-*methylthioadenine* (*Sodium Methoxide Cyclisation*).—The above "pyranose" triacetyl azo-arabinoside (13 g.) was reduced with zinc dust in acetic acid, and the product thioformylated by the method described above for the analogous triacetyl azo-ribosides. The resinous thioformamido-glycoside (8·3 g.) was cyclised by heating under reflux for 5 hours in dry ethanol (400 c.c.) containing sodium methoxide (1·6 g.). Evaporation of the solution and crystallisation of the residue from water gave a purine arabinoside (1·4 g.), m. p. 268—270°. Three further recrystallisations gave finally a product *A* (487 mg.), m. p. 273—274°, which, on periodate titration, consumed 2·67 mols./mol., unchanged by further recrystallisation (Found: C, 42·0; H, 5·1; N, 22·5. Calc. for $C_{11}H_{15}O_4N_5S$: C, 42·2; H, 4·8; N, 22·4%). From the original crystallisation mother-liquor, by concentration, a quantity of glycoside (200 mg.) was obtained which had m. p. 284° and showed a periodate uptake of 2·9 mols./mol. Recrystallisation left the m. p. unchanged and the product, which is considered to be pure 9-L-*arabopyranosido*-2-*methylthioadenine*, showed a periodate uptake of 3·03 mols./mol. (Found: C, 42·3; H, 4·9; N, 22·6. $C_{11}H_{15}O_4N_5S$ requires C, 42·2; H, 4·8; N, 22·4%); $[\alpha]_D^{17°}$ +34° ±10° (*c*, 0·058 in water).

Since product *A*, although evidently a mixture of furanose and pyranose forms, could not be separated into its components by recrystallisation, a portion (200 mg.) was acetylated by shaking for 6 hours with acetic anhydride in pyridine and keeping the solution overnight before working up. The product (265 mg.), in a small amount of chloroform, was poured on a column (15 × 1·5 cm.) of neutral alumina. The column was washed with chloroform containing methanol (1%), and the washings were collected in two arbitrary fractions. Each fraction was evaporated, and the residue dissolved in chloroform and again chromatographed in the same way, so that finally four different fractions were obtained from the original material. Each of these fractions was then treated as follows. The solvent was removed under reduced pressure, and the glassy residue dissolved in methanol (5 c.c.) containing sodium methoxide (5 mg.). The crystalline 9-L-arabinosido-2-methylthioadenine which separated was collected and submitted to periodate oxidation; succeeding fractions (in order of elution) consumed the following amounts of periodate: 2·84, 2·95, 2·89, 2·30 mols./mol. These results support the view that the original product *A* is a furanose–pyranose mixture, and suggest that, given larger amounts of material, it might well be possible to isolate the pure components; further experiments in this direction were not carried out.

Cyclisation of "Pyranose" 6-*Amino*-5-*thioformamido*-4-*triacetyl*-L-*arabinosidamino*-2-*methylthiopyrimidine with Potassium Acetate in Methyl Cyanide*.—The thioformamido-compound (607 mg.), prepared as described above starting from the condensation product of L-arabinose and 4 : 6-diamino-2-methylthiopyrimidine, was heated under reflux for 14 hours with fused potassium acetate (370 mg.) in dry methyl cyanide (50 c.c.). The resulting solution was evaporated to dryness and the residue thrice extracted with chloroform (40 c.c. in all); the combined extracts were poured on neutral alumina (35 g.; column, 3-cm. diam.), and the glycoside was washed through with more chloroform (300 c.c.). Evaporation of the pale yellow chloroform solution gave a resin which was set aside overnight in methanol (3 c.c.) with sodium methoxide (3 mg.). The 9-L-arabinosido-2-methylthioadenine which separated (72 mg.) had m. p. 273—274° and consumed 2·85 mols. of periodate per mol. in 90 hours (Found: N, 22·4. Calc. for $C_{11}H_{15}O_4N_5S$: N, 22·4%).

Cyclisation of "Furanose" 6-*Amino*-5-*thioformamido*-4-*triacetyl*-L-*arabinosidamino*-2-*methylthiopyrimidine with Potassium Acetate in Methyl Cyanide*.—5-Benzoyl 2 : 3 : 4-triacetyl L-arabinose (from 7·86 g. of the diethyl thioacetal; Part XVII, *loc. cit.*) was added to a hot solution of 4 : 6-diamino-2-methylthiopyrimidine (10·2 g.) and ammonium chloride (0·26 g.) in dry ethanol (250 c.c.). After 3 days at room temperature the solvent was removed *in vacuo*, and the residue extracted with chloroform (120 c.c. in three portions). The resin obtained on evaporating the chloroform extract was dissolved in dry methanol (100 c.c.), and sodium methoxide (0·75 g.) added. After 1 hour acetic acid (0·2 c.c.) was added and the solution poured into a neutral diazotised solution of 2 : 5-dichloroaniline (4·8 g.). The crude precipitated azo-compound was collected at once and dried at 50° *in vacuo* (15·5 g.). It was then dissolved in dry pyridine (150 c.c.) and adsorbed on activated alumina (250 g.; column, 8-cm. diam.), the column being washed with dry pyridine (400 c.c.). The upper, coloured portion of the column was removed and stirred at 0° with more pyridine, whilst acetic anhydride (75 c.c.) was added dropwise. Next day ethanol (75 c.c.) was added, and after 1 hour the alumina was extracted with ethyl acetate (6 × 150 c.c.), a little ethanol (50 c.c.) being added before the final extraction. The crude acetylated

azo-arabinoside obtained on evaporating the extracts was purified by chromatography on neutral alumina (200 g.; column, 3·5-cm. diam.) in ethyl acetate, and finally by precipitation from ethereal solution by *n*-hexane; it formed an orange-yellow powder (1·8 g.) and was directly reduced in the usual manner with zinc dust in ethyl acetate–acetic acid. The crude 5-amino-arabinoside was thioformylated in methanol, yielding the resinous 5-thioformamido-compound (400 mg.). This material was cyclised by boiling under reflux for 14 hours with fused potassium acetate (240 mg.) in dry methyl cyanide (40 c.c.). Worked up in the manner described in the preceding experiment, the cyclised material yielded a 9-L-arabinosido-2-methylthioadenine (28 mg.), m. p. 274° (Found: N, 22·4. Calc. for $C_{11}H_{15}O_4N_5S$: N, 22·4%). During 70 hours it consumed 2·3 mols. of periodate per mol.

6-*Amino*-4-(5′-*benzoyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—5-Benzoyl 2 : 3 : 4-triacetyl D-ribose (from 5 g. of the diethyl thioacetal) was added to a hot solution of 4 : 6-diamino-2-methylthiopyrimidine (6·5 g.) and ammonium chloride (0·165 g.) in dry ethanol (160 c.c.). After 40 hours the mixture was evaporated under reduced pressure, and the residue extracted with chloroform (3 × 20 c.c.), unchanged 4 : 6-diamino-2-methylthiopyrimidine (4·3 g.) being insoluble. The chloroform extract was evaporated, the residue dissolved in dry methanol (60 c.c.), and sodium methoxide (0·2 g.) added. After 1½ hours, the whole was poured into a neutral diazotised solution of 2 : 5-dichloroaniline (3·1 g.). The granular orange-yellow precipitate (10 g.) was washed, dried, and extracted with warm, dry benzene (55 c.c.); the residue (5·1 g.) consisted largely of sugar-free azo-compound. The benzene extract was poured on alumina (200 g. of Spence Grade H + 5% of water; column, 5·5-cm. diam.), and the chromatogram developed with benzene (400 c.c.). Dry ether (350 c.c.) eluted an orange band, and evaporation of the eluate gave an orange resin (1·56 g.). This resin was again dissolved in ether (50 c.c.) and chromatographed on alumina (150 g. of Spence Grade H + 2·5% of water; 5·5-cm. diam.), the column being washed with ether. The first material passing through the column was partly acetylated, but elution of the remaining dark orange band from the top of the column, with ether containing methanol (5%), and evaporation gave the *benzoyl azo-riboside* as an orange amorphous powder (0·6 g.) (Found in material dried at 70°: C, 49·1; H, 4·0; N, 15·1. $C_{23}H_{22}O_5N_6SCl_2$ requires C, 48·8; H, 3·9; N, 14·8%).

6-*Amino*-4-(5′-*benzoyl*-2′ : 3′-*diacetyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Acetic anhydride (0·5 c.c.) was added to a solution of 6-amino-4-(5′-benzoyl-D-ribofuranosidamino)-5-(2″ : 5″-dichlorobenzeneazo)-2-methylthiopyrimidine (0·5 g.) in pyridine (3 c.c.), and the mixture set aside overnight. Ethanol (0·5 c.c.) was then added and after 1 hour the whole was evaporated to dryness under reduced pressure. The residue was dissolved in benzene (20 c.c.), chromatographed on neutral alumina (75 g.; column, 3-cm. diam.) and developed with benzene (500 c.c.) and benzene–ether (5 : 1; 250 c.c.). This treatment washed out two yellow bands, and the remaining yellow band was eluted by washing with benzene–ether (3 : 1; 100 c.c.). The benzene–ether eluate gave the *benzoyl diacetyl* compound as an orange resin (0·19 g.) (Found, in material dried at 70°: C, 50·3; H, 4·5; N, 13·4. $C_{27}H_{26}O_7N_6SCl_2$ requires C, 50·0; H, 4·0; N, 12·9%). A narrow deep-orange band still remained on the column and could be eluted with ether; it consisted largely of the unacetylated starting material.

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348. *Experiments on the Synthesis of Purine Nucleosides. Part XXIII. A New Synthesis of Adenosine.*

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The extension of the general synthetic route to purine glycosides, developed in earlier papers of this series, to include furanose forms is exemplified by a new synthesis of the natural nucleoside adenosine. 2 : 3 : 4-*Triacetyl* 5-*benzyl* D-*ribose* condenses with 4 : 6-diamino-2-methylthiopyrimidine to give a Schiff base which is deacetylated with complete retention of the 5-benzyl group and then isomerises to a 5-benzylribofuranoside. By following the normal procedure of nucleoside synthesis from this point and removing the protecting benzyl residue and the 2-methylthio-group from the resulting purine glycoside with Raney nickel, 9-β-D-ribofuranosidoadenine is obtained, identical with adenosine prepared from yeast ribonucleic acid.

IN earlier papers of this series a general and unambiguous method for the synthesis of purine 9-glycosides was developed which rested on the condensation of an aldose with a suitable 4 : 6-diaminopyrimidine derivative, to give the corresponding 6-amino-4-glycosidamino-pyrimidine, and proceeded by introduction of an amino-group into position 5 of the pyrimidine ring, thioformylation, and cyclisation of the 5-thioformamido-compound so obtained, to yield the required purine glycoside. When it was found that this procedure yielded, in general, pyranose glycosides, whereas the naturally occurring nucleosides have a furanose structure, experiments were initiated with the object of so modifying the initial linkage of sugar to pyrimidine that 6-amino-4-glycofuranosidamino-pyrimidines would be produced; some of these

experiments have already been reported (Part XVII, Kenner, Lythgoe, and Todd, *J.*, 1948, 957). The work carried out during the establishment of the general route to the purine glycopyranosides had, however, not only finally established the structure of the purine and pyrimidine ribonucleosides (Davoll, Lythgoe, and Todd, *J.*, 1946, 833; Howard, Kenner, Lythgoe, and Todd, *ibid.*, p. 861; Lythgoe, Smith, and Todd, *J.*, 1947, 355), but had also made it possible, after the preparation of acetohalogenoribofuranoses (Howard, Lythgoe, and Todd, *J.*, 1947, 1052), to synthesise the naturally occurring nucleosides (Howard, Lythgoe, and Todd, *loc. cit.*; Davoll, Lythgoe, and Todd, *J.*, 1948, 967, 1685) by extension of the methods used earlier for pyrimidine glucosides by Hilbert and Johnson (*J. Amer. Chem. Soc.*, 1930, **52**, 4489) and for adenine glucoside by Fischer and Helferich (*Ber.*, 1914, **47**, 210). Xanthosine was also synthesised by application of the Hofmann degradation to 3-triacetyl-D-ribofuranosidoglyoxaline-4 : 5-dicarboxyamide (Howard, McLean, Newbold, Spring, and Todd, this vol., p. 232).

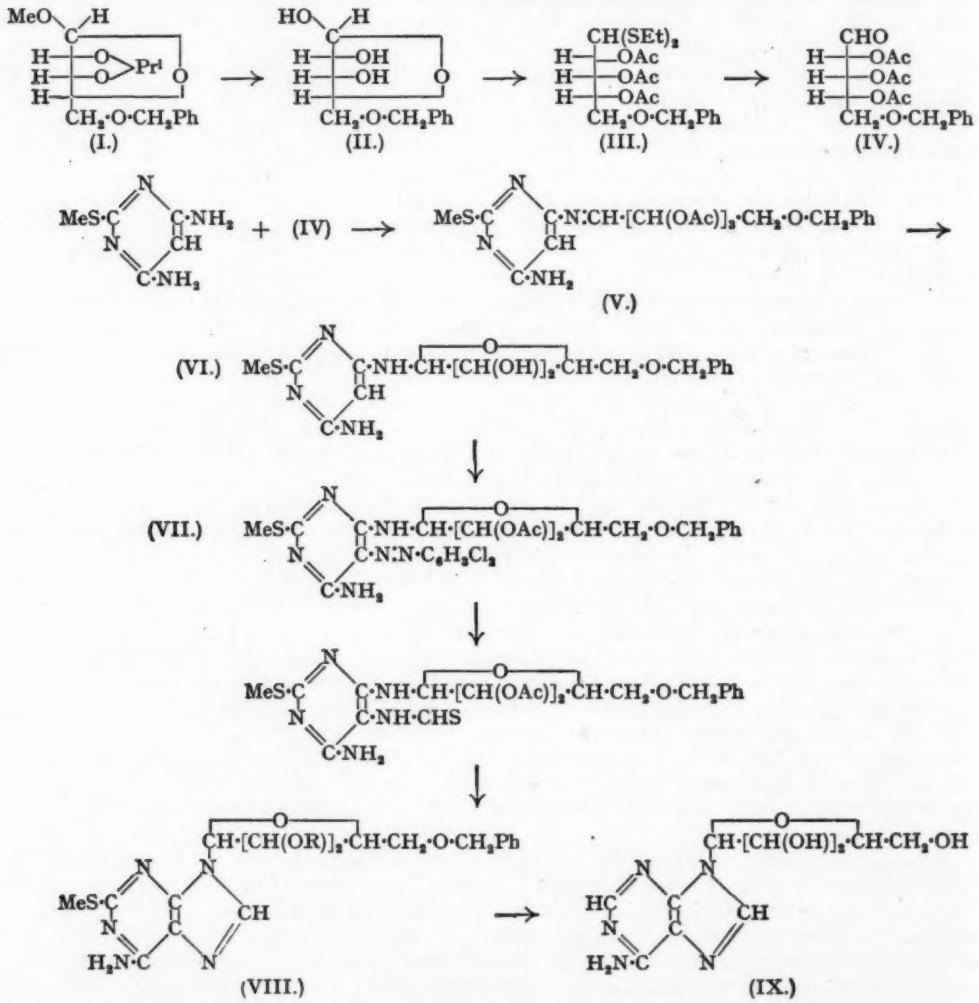
These syntheses, although valuable in individual cases, are of limited application and their accomplishment did not in any way reduce the importance of extending our general synthetic procedure to cover the 9-glycofuranosidopurines, since such a method is an essential part of the scheme of investigations on nucleosides and nucleotides upon which we are engaged. The successful extension of the general procedure to the synthesis of adenosine forms the subject of the present memoir.

Initial attempts to prepare 6-amino-4-pentofuranosidaminopyrimidines by condensation of 5-benzoyl 2 : 3 : 4-triacetyl pentoses with 4 : 6-diaminopyrimidines to give Schiff bases, followed by hydrolytic removal of the acetyl groups and consequent isomerisation to 5′-benzoylpentofuranosides, were only partly successful. The unsatisfactory results were due, in part, to the fact that some debenzoylation always occurred as well as deacetylation, even under the mildest conditions, and, in part, to other complications arising from furanose–pyranose interconversion and α–β-isomerisation at subsequent stages in the syntheses. These investigations have been described elsewhere in detail (Kenner, Lythgoe, and Todd, *J.*, 1948, 957; also, preceding paper) and led to the conclusion that the difficulty of preparing adequate quantities of pure furanosidamino-pyrimidines by this means was so great that the use of a benzoyl residue as a protecting group in position 5 of the pentose residue should be abandoned. It was evident, however, from the work which had been done, that the Schiff-base route to purine glycofuranosides was sound in principle, and that it could be successfully applied if the 5-position in the *aldehydo*-sugar derivative were protected by a group which could be retained intact until the glyoxaline ring of the purine had been closed and furanose–pyranose interconversion thereby prevented. For this purpose we elected to use a benzyl residue, since it would undoubtedly be retained under conditions bringing about complete removal of acetyl groups and could be removed finally by hydrogenation.

5-Benzyl 2 : 3-iso*propylidene methyl*-D-*ribofuranoside* (I) was prepared from 2 : 3-*iso*propylidene methyl-D-ribofuranoside (Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 301) by benzyl chloride and potassium hydroxide in xylene; it was also obtained, in lower yield, by adding benzyl chloride to a solution of the riboside and sodium in liquid ammonia. Hydrolysis with dilute acid yielded *5-benzyl* D-*ribofuranose* (II) as a syrup, whose structure was confirmed by acetylation followed by removal of the benzyl group by hydrogenolysis; acetylation of the product so obtained gave the known 1 : 2 : 3 : 5-tetra-acetyl D-ribofuranose. From 5-benzyl D-ribofuranose, 2 : 3 : 4-*triacetyl 5-benzyl* D-*ribose diethyl thioacetal* (III) was obtained by interaction with ethanethiol in presence of hydrochloric acid, followed by acetylation, and from it 2 : 3 : 4-*triacetyl 5-benzyl* D-*ribose* (IV) was prepared as a colourless syrup by the normal procedure.

Condensation of (IV) with 4 : 6-diamino-2-methylthiopyrimidine in ethanol containing a little ammonium chloride gave a glassy product, presumably the Schiff base (V), which was deacetylated with methanolic ammonia, to convert it into the required glycoside (VI), and coupled with diazotised 2 : 5-dichloroaniline in the usual way. The crude azo-compound was acetylated and the 6-*amino*-4-(2′ : 3′-*diacetyl*-5′-*benzyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (VII) purified by chromatography. Complete separation of (VII) from a small amount of adhering sugar-free material (*ca.* 10%) is rather tedious, but for use in the next step complete removal of the latter is not essential since sugar-free impurities are readily eliminated in the later stages. Model experiments having shown that the benzyl group in 5-benzyl pentoses is not affected by zinc dust and acetic acid, (VII) was reduced with this reagent, and the resulting 5-amino-compound directly thioformylated. The resinous thioformamido-glycoside so obtained was cyclised using sodium methoxide, but the purine glycoside (VIII; R = H) showed no tendency to crystallise. It was, however, clear that the most direct proof of the validity of the synthesis would be the production of adenosine (IX) itself, whose

structure is known. It was, therefore, decided to proceed directly to adenosine without isolating (VIII; R = H) in a pure state. The resinous material was acetylated and the acetyl derivative



(VIII; R = Ac) heated under reflux in ethanol with Raney nickel to effect simultaneous removal of the benzyl and the methylthio-group. Deacetylation of the product furnished adenosine (9-β-D-ribofuranosidoadenine) (IX), identical with a sample of the natural nucleoside prepared by hydrolysis of yeast ribonucleic acid.

The synthesis of adenosine in this way establishes the validity of the Schiff-base route, and it is clear that it should be generally applicable to other purine glycofuranosides, including those derived from deoxypentoses, provided that the appropriate 5-benzyl sugar derivatives can be made available.

Experimental.

5-Benzyl 2 : 3-iso*Propylidene Methyl-*D*-ribofuranoside.—Method I.* 2 : 3-*iso*Propylidene methyl-D-ribofuranoside (5 g.; Levene and Stiller, *loc. cit.*) was dissolved in dry xylene (50 c.c.), finely powdered potassium hydroxide (20 g.) and benzyl chloride (4 g.) were added, and the mixture was stirred at 80° for 4 hours. Water was now added, the mixture evaporated *in vacuo*, and the residue extracted with ether. The ethereal extract was dried over sodium sulphate, the ether removed, and the residue distilled in a high vacuum. Aftr traces of benzyl chloride and benzyl alcohol had been removed at a bath temp. of 50°, the *riboside* distilled at 95—100° (bath temp.)/10^{-4} mm. as a colourless syrup (6 g.), $[\alpha]_D^{17°}$ −36° ±2° (*c*, 1·3 in chloroform) (Found: C, 65·3; H, 7·8. $C_{16}H_{22}O_5$ requires C, 65·3; H, 7·5%).

Method II. 2 : 3-*iso*Propylidene methyl-D-ribofuranoside (2 g.) in dry ether (1 c.c.) was added to liquid ammonia (60 c.c.). Sodium (0·25 g.) was added to the solution, and the mixture stirred until the

sodium dissolved and the blue colour disappeared. Benzyl chloride (1·7 c.c.) was now added, and the ammonia was allowed to evaporate. Dry air was blown through the flask for 2 hours, the residue dried over phosphoric oxide and extracted with ether, the extract evaporated, and the product distilled as above. The yield was 1·6 g. (Found: C, 66·0; H, 7·6%).

5-Benzyl D-*Ribofuranose*.—5-Benzyl 2 : 3-*iso*propylidene methyl-D-ribofuranoside (2·3 g.) was heated on a boiling water-bath for 3 hours with 50% aqueous ethanolic hydrochloric acid (50 c.c. of 0·05N). The acid was neutralised with barium carbonate, and the filtered solution evaporated under reduced pressure. The residue was extracted with chloroform, the extract washed with a little water, dried (Na_2SO_4), and evaporated. The *product* (1·6 g.) was a pale yellow syrup which could not be distilled or crystallised; it had $[\alpha]_D^{18°}$ −8·5° (*c*, 1·5 in alcohol) (Found: C, 59·6; H, 6·9. $C_{12}H_{16}O_5$ requires C, 60·0; H, 6·7%).

A sample of 5-benzyl D-ribofuranose (2 g.) prepared in this way was acetylated in the usual manner with acetic anhydride (6 c.c.) in pyridine (12 c.c.). The syrupy acetyl compound (2·7 g.) so obtained was dissolved in ethanol and hydrogenated under atmospheric pressure using a palladised charcoal catalyst. Catalyst was removed by filtration, the solution evaporated, and the residue acetylated with acetic anhydride (2 c.c.) in pyridine (6 c.c.). The product was purified by distillation under reduced pressure and finally by recrystallisation from ethanol, whereupon 1 : 2 : 3 : 5-tetra-acetyl D-ribofuranose (1 g.) was obtained, having m. p. 57—58°, undepressed in admixture with an authentic specimen (m. p. 58°; Howard, Lythgoe, and Todd, *J.*, 1947, 1052).

2 : 3 : 4-*Triacetyl 5-Benzyl* D-*Ribose Diethyl Thioacetal*.—5-Benzyl D-ribofuranose (3·8 g.) was dissolved in a mixture of dioxan (0·25 c.c.) and concentrated hydrochloric acid (5 c.c.; *d* 1·19), the solution cooled to 0°, and ethanethiol (3 c.c.) added in three portions with shaking. After being shaken for a further 10 minutes the mixture was set aside at room temperature for 15 minutes and then poured into excess of ice-cold saturated aqueous sodium hydrogen carbonate, and the whole extracted with chloroform. The chloroform extracts, dried (Na_2SO_4) and evaporated, gave crude 5-benzyl D-ribose diethyl thioacetal as a yellow syrup (3 g.) which was acetylated in the usual way with acetic anhydride (10 c.c.) in pyridine (30 c.c.). The product distilled at 170—180° (bath temp.)/10^{-4} mm. as a colourless syrup (1·3 g.) which did not crystallise; it had $[\alpha]_D^{18°}$ +9° (*c*, 0·87 in chloroform) (Found: C, 56·4; H, 7·1. $C_{22}H_{32}O_7S_2$ requires C, 55·9; H, 6·8%).

2 : 3 : 4-*Triacetyl 5-Benzyl* D-*Ribose*.—2 : 3 : 4-Triacetyl 5-benzyl D-ribose diethyl thioacetal (3·7 g.) was dissolved in a mixture of acetone (40 c.c.) and water (4 c.c.), yellow mercuric oxide (8 g.) was added, and the mixture stirred vigorously while a solution of mercuric chloride (9 g.) in acetone (20 c.c.) was run in during 1 hour. Stirring was continued for 24 hours at room temperature, and the mixture filtered. The filtrate was evaporated under reduced pressure, and traces of water removed from the residual syrup by distillation with benzene. The residue was extracted with chloroform (3 × 50 c.c.), and the extract washed with 40% aqueous potassium iodide (50 c.c.) and then with water and dried (Na_2SO_4). Chloroform was removed by evaporation, and the aldehydo-*sugar* purified by distillation at 150° (bath temp.)/10^{-3} mm. It formed a colourless syrup (2·6 g.), $[\alpha]_D^{17°}$ −4·2° ±0·8° (*c*, 0·65 in chloroform) (Found: C, 59·4; H, 6·4. $C_{18}H_{28}O_8$ requires C, 59·0; H, 6·0%).

6-*Amino*-4-(2′ : 3′-*diacetyl*-5′-*benzyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Ammonium chloride (0·3 g.), 4 : 6-diamino-2-methylthiopyrimidine (11·4 g.), and 2 : 3 : 4-triacetyl 5-benzyl D-ribose (6·6 g.) were dissolved in boiling absolute ethanol (270 c.c.), and the solution was kept at room temperature for 24 hours and then evaporated under reduced pressure. The residue was extracted with chloroform, unchanged diamine remaining undissolved, and the extract on evaporation yielded a yellowish glass (8·8 g.). This crude Schiff base was dissolved in methanol (200 c.c.) previously saturated with ammonia at 0°, and the solution set aside for 2 days and then evaporated, the deacetylated glycoside being thus obtained as a syrup.

A portion of this syrup (4·3 g.) was dissolved in pyridine (100 c.c.) and poured into a neutral diazotised solution of 2 : 5-dichloroaniline (1·85 g.); after 2 hours at 0° the solution was diluted with water (600 c.c.), and the precipitated azo-compound (4·5 g.) collected, washed with water, and dried. The dried material was acetylated in the cold with acetic anhydride (5 c.c.) in pyridine (10 c.c.), and the product chromatographed on neutral alumina (140 g.; column, 4-cm. diam.), the column being washed with benzene, then with benzene containing increasing amounts of chloroform, and finally with chloroform alone. The main bulk (1·1 g.) of the product was eluted by washing with chloroform (900 c.c.) containing 0·5% of methanol. Evaporation of the eluate, followed by dissolution in ethanol and precipitation with hexane, gave an amorphous orange-yellow product which, from its analysis, appeared to be the desired azo-glycoside contaminated with *ca.* 10—12% of sugar-free azo-compound (Found: C, 50·1; H, 4·1; N, 15·0%). In order to obtain a pure specimen, the above material was deacetylated with sodium methoxide in the usual manner and chromatographed on neutral alumina, the column being thoroughly washed with ethyl acetate. The washed column was extruded and the adsorbed material acetylated directly with cold acetic anhydride in pyridine solution. The acetylated product was adsorbed on a column of neutral alumina, and the *azo-glycoside* eluted by washing with ethyl acetate. Purified by precipitation from ethanolic solution with hexane, it was obtained as an orange-yellow powder which softened and melted at *ca.* 90° (Found: C, 51·2; H, 4·0; N, 13·4. $C_{27}H_{28}O_6N_6SCl_2$ requires C, 51·0; H, 4·4; N, 13·2%), $[\alpha]_D^{16°}$ +660° ±60° (*c*, 0·048 in chloroform).

Adenosine (9-β-D-*Ribofuranosidoadenine*).—The above acetyl azo-riboside (1 g. containing *ca.* 12% of sugar-free material) and zinc dust (8 g.) were stirred vigorously in boiling ethyl acetate (80 c.c.), and a mixture of acetic acid (4 c.c.) in ethyl acetate (40 c.c.) was added dropwise during 30 minutes. The solution was decanted from zinc, the latter twice extracted with warm ethyl acetate, and the combined solution and extracts were evaporated to dryness under reduced pressure (nitrogen). The resin obtained was triturated with light petroleum (b. p. 60—80°), and the insoluble portion dissolved in methanol (100 c.c.) and cooled to 0°. An ice-cold solution of sodium dithioformate (0·8 g.) in methanol (5 c.c.) was added, followed by acetic acid (0·2 g.) in methanol (5 c.c.), and the mixture set aside for 4 hours. A further quantity of dithioformic acid (from 0·4 g. of sodium dithioformate) was added in the same way, and the whole kept overnight and then heated under reflux for 1 hour. Evaporation under reduced pressure gave a residue which was extracted thoroughly with ethyl acetate, and the extracts were

evaporated. The solid residue was dissolved in hot benzene (50 c.c.) and adsorbed on a column of neutral alumina (30 g.; 2-cm. diam.). The column was washed with benzene until the washings were colourless, and the thioformamido-glycoside then eluted with pyridine (100 c.c.); evaporation of the eluate gave a brittle resin (0·3 g.) which could not be crystallised.

The resinous thioformamido-glycoside was heated under reflux in absolute ethanol (20 c.c.) with sodium methoxide (60 mg.) under nitrogen for 4 hours. The resulting solution was neutralised with acetic acid and evaporated to dryness, giving the crude purine glycoside as a brownish resin, which did not crystallise. It was accordingly acetylated by shaking it for 24 hours with acetic anhydride (1 c.c.) in pyridine (5 c.c.), the mixture being worked up in the usual manner. The product was dissolved in ethanol (30 c.c.), Raney nickel (3 g.) added, and the whole boiled for 2 hours and filtered. The nickel was extracted (Soxhlet) with a mixture of ethanol, water, and pyridine (90 : 5 : 5) for 12 hours, and the extract combined with the ethanolic filtrate. Evaporation yielded a glassy residue which was deacetylated with sodium methoxide (15 mg.) in methanol (15 c.c.). The methanol was distilled off and replaced by water (10 c.c.), acetic acid being added to neutrality. When this solution evaporated slowly at room temperature under reduced pressure, a quantity of crystalline material (largely sugar-free purine contaminated with some nickel complex) separated and was filtered off. Hot saturated aqueous picric acid was added to the filtrate. Adenosine picrate separated and, after two recrystallisations from water, formed yellow needles (30 mg.), m. p. 181—185° (decomp.), alone or mixed with an authentic specimen (m. p. 181—185°) (Found: C, 38·5; H, 3·4; N, 22·3. Calc. for $C_{10}H_{13}O_4N_5,C_6H_3O_7N_3$: C, 38·7; H, 3·2; N, 22·6%).

To prepare adenosine itself, the picrate (100 mg.) was acetylated in the usual way with acetic anhydride (0·3 c.c.) in pyridine (1·5 c.c.), and the product dissolved in chloroform and decomposed by adsorption on neutral alumina. The picric acid-free product was eluted with chloroform (100 c.c.) containing 1% of ethanol and deacetylated with sodium methoxide (10 mg.) in methanol (2 c.c.). Adenosine crystallised directly from the hydrolysis solution and was recrystallised from water (yield, 38 mg.). It had m. p. 230—231°, undepressed in admixture with an authentic specimen (m. p. 230—231°) from natural sources, and had $[\alpha]_D^{18°}$ −62·1° (*c*, 0·34 in water) (Found, in material dried at 100° for 12 hours: C, 45·0; H, 4·8; N, 26·3. Calc. for $C_{10}H_{13}O_4N_5$: C, 45·0; H, 4·9; N, 26·2%). On periodate titration the uptake of oxidant in 48 hours was 0·98 mol./mol.

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349. *The Replacement of the Diazonium by the Nitro-group. Part V. New Preparations of* 2 : 3- *and* 2 : 5-*Dinitrotoluene from Monoamines and the Simultaneous Introduction of Two Nitro-groups into Some Homonuclear Diamines.*

By HERBERT H. HODGSON and FRED HEYWORTH.

2 : 3- and 2 : 5-Dinitrotoluene are more readily prepared by the procedure described by Hodgson, Heyworth, and Ward (*J.*, 1948, 1512) from 3- and 5-nitro-*o*-toluidine than by previously described methods. The same procedure permits the preparation of the dinitro-compounds from bis-diazotised *m*- and *p*-phenylene- and *m*- and *p*-tolylene-diamines.

PREVIOUSLY reported methods for the preparation of 2 : 5-dinitrotoluene in moderately large quantities such as the oxidation of 2 : 5-dinitrosotoluene with cold fuming nitric acid (Fierz-David and Sponagel (*Helv. Chim. Acta*, 1943, **26**, 98), the nitration of *m*-nitrotoluene (Sirks, *Rec. Trav. chim.*, 1908, **27**, 211), and the reaction of nitrogen peroxide with *p*-toluquinone dioxime in ether (Olivieri-Tortovici, *Gazzetta*, 1900, **30**, I, 534), were found to be unsuitable. Grell's Sandmeyer procedure (*Ber.*, 1895, **28**, 2565), modified by neutralising the solution of 5-nitrotoluene-2-diazonium sulphate with calcium carbonate and subsequently decomposing it with cupro–cupri sulphite (Hodgson, Heyworth, and Ward, *loc. cit.*) gave a 52% yield of pure 2 : 5-dinitrotoluene.

The route from 5-nitro-*o*-toluidine was preferred to that from 6-nitro-*m*-toluidine. Nitration of aceto-*o*-toluidide and hydrolysis of the product gave a mixture of 3- and 5-nitro-*o*-toluidine, from which the 3-isomer was removed by steam-distillation of the mixed hydrochlorides. Preparation from aceto-*m*-toluidide, whereby the required 6-nitro-*m*-toluidine had to be separated from two accompanying isomers, was not so satisfactory. The initial nitration was less efficient than in the case of acet-*o*-toluidide and the conversion of the amino- into the nitro-group did not give such a good yield.

There appears to be no evidence in the literature of the simultaneous replacement of two

diazo- by two nitro-groups in the same aromatic nucleus. The solid bisdiazonium sulphates of *m*- and *p*-phenylene- and *m*- and *p*-tolylene-diamines were prepared by the method of Hodgson and Mahadevan (*J.*, 1947, 325) and their aqueous solutions added to excess of aqueous sodium nitrite containing a suspension of cupro-cupri sulphite as catalyst, whereupon the corresponding dinitro-compounds were obtained in small yield. Failure, however, was experienced with *o*-phenylenediamine, 4-nitro-*o*-phenylenediamine, and 1 : 8-naphthylenediamine. When the amino-groups were in different nuclei, as in benzidine, the simultaneous replacement by two nitro-groups occurred in 27% yield (cf. Hodgson, Mahadevan, and Ward, *J.*, 1947, 1392, who give 16%).

Experimental.

2 : 5-*Dinitrotoluene*.—(a) *From aceto-*o*-toluidide*. To aceto-*o*-toluidide (24 g.) in sulphuric acid (50 c.c., *d* 1·84), nitric acid (10 c.c., *d* 1·42) in sulphuric acid (10 c.c., *d* 1·84) was gradually added. The nitration was initiated by the addition of 2 c.c. of the mixed acids to the toluidide solution at 60° together with a trace of sodium nitrite, whereupon the temperature rapidly rose to 70°. Thereafter the mixture was maintained at 30—40° during the addition of the remainder of the mixed acids. By this means, the nitration was carried out at as low a temperature as possible, and the production of 3-nitroaceto-*o*-toluidide kept at a minimum. After being stirred for a further hour to ensure completion of the nitration, the mixture was poured into iced water (2 l.), and the yellow mixture of solid 3- and 5-nitroaceto-*o*-toluidides (29 g.; 90% yield) filtered off and washed with cold water, aqueous sodium hydrogen carbonate, and finally with cold water. The solid was then heated under reflux for 3 hours with sulphuric acid (100 c.c., *d* 1·84) and water (300 c.c.), and the hot orange solution filtered and cooled, whereupon crude 5-nitro-*o*-toluidine crystallised. The mother liquor after being made just alkaline with 20% sodium hydroxide, gave a mixture of 3- and 5-nitro-*o*-toluidine, which was added to hydrochloric acid (30 c.c., *d* 1·18) in water (100 c.c.). 3-Nitro-*o*-toluidine (1·7 g.) was removed by steam distillation; the filtered residual liquor gave crystalline 5-nitro-*o*-toluidine on cooling (total yield, 18·6 g.) 5-Nitro-*o*-toluidine (5 g.) was diazotised in sulphuric acid (10 c.c., *d* 1·84), water (10 c.c.), and ice (10 g.), by addition at 0° of sodium nitrite (2 g.) in water (10 c.c.). The solution of the diazonium sulphate was poured on to calcium carbonate (15 g.) and a little ice, the mixture filtered, and the clear solution stirred into one of sodium nitrite (20 g.) in water (100 c.c.) containing freshly prepared cupro-cupri sulphite [made by the addition of crystallised copper sulphate (10 g.) in water (100 c.c.) to crystalline sodium sulphite (10 g.) in water (50 c.c.)]. The temperature rose to 30—40°, and nitrogen was evolved. When this had ceased, the mixture was steam-distilled and 2 : 5-dinitrotoluene (3·1 g., 52% yield), m. p. 49—51°, was obtained from the distillate. In further experiments yields of 48, 50, and 50% were obtained.

(b) *From aceto-*m*-toluidide*. This nitration was particularly difficult to start and afterwards became exceedingly violent and produced only tarry products, so that the initiation procedure had to be carried out at 70°. The mixture was then rapidly cooled to 20—30° and kept at that temperature until all the mixed acid had been added. Using the same quantities as for aceto-*o*-toluidide, a mixture of nitroaceto-*m*-toluidides (29 g.; 90% yield) was obtained, which was separated by the technique described above into 6-nitro-*m*-toluidine (12·8 g.), and a mixture of 2- and 4-nitro-m-toluidines (1·6 g.).

Nitro-compounds were obtained by the cupro-cupri sulphite procedure: 2 : 5-dinitrotoluene (2·5 g.; 40% yield) from 6-nitro-*m*-toluidine (5 g.), 3 : 4-dinitrotoluene (2·7 g.; 45% yield) from 4-nitro-*m*-toluidine (5 g.), and 3 : 4-dinitrotoluene (3·0 g., 50% yield) from *m*-nitro-*p*-toluidine (5 g.) (identical with the material prepared from 4-nitro-*m*-toluidine).

2 : 3-*Dinitrotoluene*.—2 : 3-Dinitrotoluene (3·0 g., 50%) was obtained from 3-nitro-*o*-toluidine (5 g.) by the procedure given under (a) above.

Simultaneous Replacement of Two Diazonium by Two Nitro-groups.—A cooled solution of the diamine (5 g.) in glacial acetic acid (50 c.c.) was added to one of sodium nitrite (7 g.) in sulphuric acid (40 c.c., *d* 1·84) at 0°, the mixture was warmed gradually from 0° to 20—30° during 30 minutes and then stirred in a thin stream into ice-cold dry ether (200 c.c.), whereupon crystals of the bisdiazonium sulphate (together with anhydrous sodium sulphate) separated. After being left for 15 minutes the mixture was filtered and the crystals washed with dry ether (20 c.c.), dissolved in iced water (30 c.c.), and added at 30° to a solution of sodium nitrite (30 g.) in water (50 c.c.) containing cupro-cupri sulphite (10 g.) as catalyst. *m*-Dinitrobenzene (0·4 g. from 5 g. of *m*-phenylenediamine) and *p*-dinitrobenzene (2·0 g., 25% yield from 5 g. of *p*-phenylenediamine) were obtained by steam-distillation of the crude products. Attempts to prepare picric acid from 4 : 6-dinitrobenzene-1 : 2-diazo-oxide, and 2 : 6-dinitro-1-naphthol from 6-nitronaphthalene-1 : 2-diazo-oxide were unsuccessful.

*Some additional and improved preparations using the technique described above for 5-nitro-*o*-toluidine.*

Amine diazotised.	% Yield of nitro-compound.	Amine diazotised.	% Yield of nitro-compound.
Aniline	68	3-Nitro-*o*-toluidine	50
m-Nitroaniline	45	4-Nitro-*o*-toluidine	55
o-Toluidine	27	4-Nitro-*m*-toluidine	45
p-Toluidine	49	6-Nitro-*m*-toluidine	50
o-Chloroaniline	81	2-Nitro-*p*-toluidine	42
m-Chloroaniline	89	3-Nitro-*p*-toluidine	50
p-Chloroaniline	70	β-Naphthylamine	20
o-Anisidine	24	Benzidine	27
p-Anisidine	28	2 : 2′-Dinitrobenzidine	13

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350. *The Oxidation of Monoethenoid Fatty Acids and Esters. Union of Gaseous Oxygen with Elaidic Acid, and Methyl and* n-*Propyl Elaidate.*

By J. H. SKELLON and M. N. THRUSTON.

The catalytic autoxidation, at various temperatures, of highly purified elaidic acid and methyl and *n*-propyl elaidate has been studied, and the course of the complex changes occurring has been investigated. Dimerisation occurs at high temperatures (120°) on prolonged oxidation, but at lower temperatures elaidic acid and its simple esters behave during controlled oxidations in much the same way as oleic acid. The results of the autoxidations at various temperatures up to 120° are illustrated graphically, and the nature of the curves shows that peroxide formation and subsequent decomposition are a characteristic feature of these autoxidations. The composition of the products depends to a considerable extent on the conditions. The presence of terminal alkyl groups apparently favours the entry of oxygen into the monoethenoid molecule.

OXIDATION of monoethenoid systems by gaseous oxygen has been extensively studied for a large range of compounds, but comparatively little work has been devoted to *trans*-oleic acid. Hilditch (*J.*, 1926, 1828) prepared methyl elaidate and subjected it to oxidation in acetic acid with hydrogen peroxide. Ellis (*Biochem. J.*, 1932, **26**, 2315) autoxidized elaidic acid in the presence of its cobalt salt, following this later (*ibid.*, 1936, **30**, 753) by work on the oxido-acid. King (*J.*, 1939, 974) and Dorée and Pepper (*J.*, 1942, 477) oxidized the acid with hydrogen peroxide in acetic acid; Sutton (*J.*, 1944, 242) oxidized the methyl ester at 35° in the presence of ultraviolet light and showed that the primary product is apparently a hydroperoxidic ester.

Little work has been done on the catalytic autoxidation of elaidic acid and its esters at varying temperatures, and our object was to ascertain whether the course of oxidation was similar to that of oleic acid (Atherton and Hilditch, *J.*, 1944, 195; Gunstone and Hilditch, *J.*, 1945, 836) and whether such course was influenced by the nature of the terminal groups of the esters. Skellon (*J.*, 1948, 343) investigated the course of thermal catalytic oxidation of methyl oleate as an extension of previous work on the acid (*J. Soc. Chem. Ind.*, 1931, **50**, 382T) and this work, together with that cited above, gives a fair background for comparison.

Pure oleic acid was prepared as formerly described (Skellon, *loc. cit.*, p. 131T), converted into elaidic acid by nitrous fumes, and crystallised from aqueous alcohol. Although later work (Smith, *J.*, 1939, 974) has shown that low-temperature crystallisation from acetone is at least as good as, if not superior to, the lithium-salt method, the product obtained by the latter method was of very satisfactory purity.

The conditions of oxidation were arranged to give high interim peroxide formation with a minimum of fission or polymerisation of the carbon chain, and the results obtained have yielded useful data for comparison with the work of Gunstone and Hilditch (*loc. cit.*). At 55° there was a definite induction period before peroxide formation; this period was far less marked, but definite, at 85°, whilst at 120° no such phenomenon was observed. In contrast, Gunstone and Hilditch (*loc. cit.*) observed a definite induction during oxidation of methyl oleate at 120°. The rapid increase of peroxidation, followed by a very similar rate of decrease (Fig. 2), at the higher temperatures was similar to that observed by Gunstone and Hilditch, and it was apparent that at higher temperatures the onset of peroxidation was considerably favoured.

The highest peroxide values were obtained at 85°, *viz.*, 1·71% for methyl elaidate at 85° (this work) and 1·95% for methyl oleate at 80° (Gunstone and Hilditch, *loc. cit.*) (peroxide values are expressed as % of active oxygen). The observed peroxidic oxygen content was below the theoretical maximum (calc. for methyl esters, 4·88%), and the peroxides were apparently unstable to heat, decomposing rapidly into secondary oxidation products. At the higher temperatures, catalytic oxidation of the esters resulted in ultimate formation of carbonyl compounds; however, at lower temperatures and in catalytic oxidations of the parent acid, the amount of such compounds appeared to be small.

Evolution of carbon dioxide commenced some hours before maximum peroxide formation, but the small amount evolved indicated little or no major fission of the molecule. The increased

molecular weights at higher temperature (120°) indicated that when oxidation was prolonged for more than 20 hours some form of polymerisation commenced. At lower temperatures,

FIG. 1.

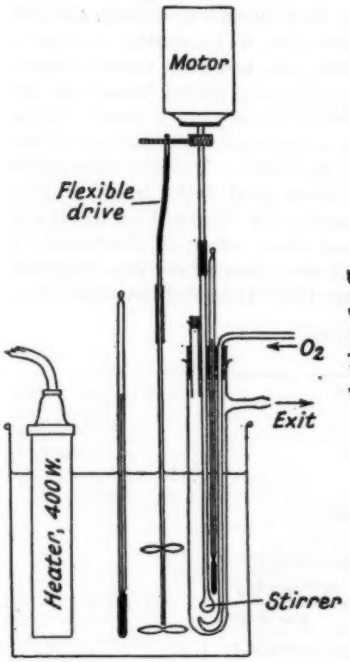


FIG. 2.

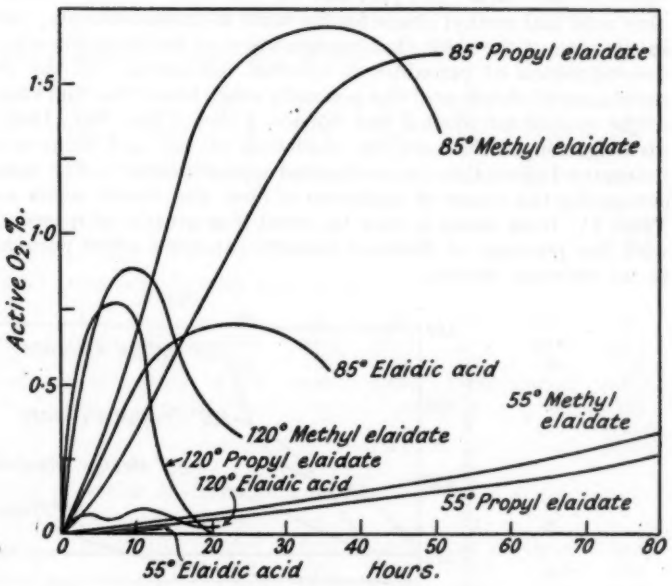
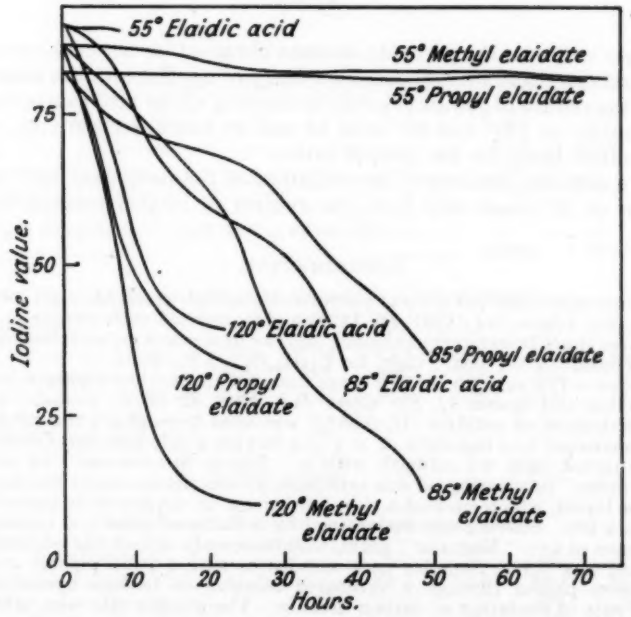


FIG. 3.



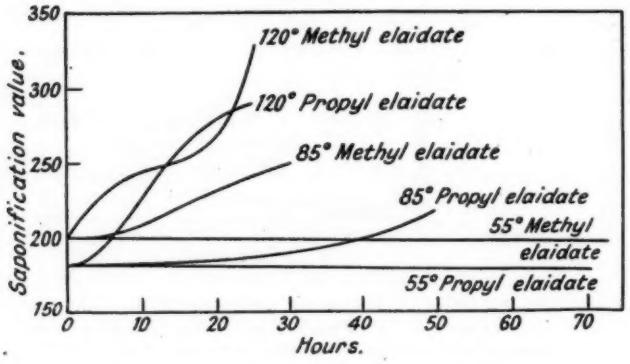
carbon dioxide evolution was very small and there was little change in peroxide and iodine values (Figs. 2 and 3). In the case of the esters, there was a marked increase in saponification value together with an irregular decrease in iodine value (Fig. 3); the observed form of the

decreases at 85° differed somewhat from that at higher temperatures, and oxygen absorption at 85° apparently occurs in stages.

These observations appear to indicate possible differences in the nature of thermal oxidations taking place at high and moderate temperature.

The course of catalytic oxidation of elaidic acid and its esters, as indicated by changes in acid value, iodine value, and peroxide oxygen content, appeared generally to be similar to that of oleic acid and methyl oleate for the same metallic catalysts; there was, however, some evidence in the case of the acids that configuration of the molecule may affect the initial formation and decomposition of peroxides in thermal oxidations, for the peroxidic-oxygen content during oxidations of elaidic acid was generally much lower than the values obtained in similar oxidations of the *cis*-acid (cf. Skellon and Spence, *J. Soc. Chem. Ind.*, 1948, **67**, 365T). Further exhaustive investigation of the catalytic oxidation of *cis*- and *trans*-monoethenoid fatty acids will be necessary before this can be decided with certainty. The necessity for identical conditions in comparing the course of oxidation of oleic and elaidic acids and their esters is illustrated in Table IV, from which it may be noted that greatly increased rates of flow of oxygen, together with the presence of different metallic catalysts, affect peroxide formation and decomposition to an extreme degree.

FIG. 4.



Finally, the lower value for the peroxide maxima obtained for the acid, as compared with the esters, indicated that esterification influences the entry of oxygen into the molecule. There was also evidence that the nature of the alkyl group in esters of either acid exerts a specific influence; the fall in iodine value, at 120° and 85° over 24 and 48 hours, respectively, was considerably greater for the methyl than for the propyl ester.

The results of a detailed systematic investigation of the numerous and varied products of catalytic oxidation at all stages will form the subject of further communications.

EXPERIMENTAL.

Elaidic Acid.—Pure oleic acid (90 g.) was prepared from high-grade olive oil (500 g.) by a method described earlier (*J. Soc. Chem. Ind.*, 1931, **50**, 130T) and isomerised with nitrous fumes. The product was recrystallised three times from aqueous alcohol, and the pure white crystals were dried over sulphuric acid; m. p. 43·6° (Found: I V., 88·4. Calc. for $C_{18}H_{34}O_2$: I V., 90·1).

Catalytic Oxidations.—The substance (15 g.) was mixed with uranium elaidate (0·0246 g.), prepared as described by Skellon and Spence (*J. Soc. Chem. Ind.*, 1948, **67**, 365T; Found: U, 30·5. Calc.: U, 28·6%). This concentration of catalyst (U, 0·05%) was used throughout the subsequent oxidations. The mixture was transferred to a test-tube (6 × 1 in.), having a side-arm and fitted as shown in Fig. 1 into an electrically-heated (400 w.) oil-bath with a "Sunvic Simmerstat" as control. As the oil temperature ("Castrolite" lubricating oil was satisfactory) was always above the m. p. of the acid, the reaction mixture was liquid, which enabled a slow fine stream of oxygen to be passed in through a thin tube drawn out into a jet. Stirring was maintained by a flattened glass rod connected to an electric motor which, by means of two "Meccano" gears, simultaneously stirred the oil-bath. The maximum temperature variation was ±1°. Samples were withdrawn with a 1-ml. pipette at suitable intervals. The exit vapours were passed through a saturated solution of barium hydroxide to indicate the commencement and rate of evolution of carbon dioxide. The oxygen rate was, arbitrarily, 50 ml. per minute, and the gas appeared as a fine stream of bubbles in the reaction vessel.

The experiments were conducted at 55°, 85°, and 120°. Changes in acid value, peroxide value, and iodine value were investigated, together with molecular weight (Rast), refractive indices (for the esters only), and qualitative tests for reducing substances in the end-products. The results are set out in Tables I, II, and III, peroxide values being expressed as % of active oxygen as determined by the

method of Skellon and Wills (*Analyst*, 1948, **73**, 78). The course of the various oxidations is illustrated in the curves in Figs. 2, 3, and 4.

Methyl Elaidate.—Elaidic acid (36 g.) was treated with "AnalaR" methyl alcohol (140 ml.) and conc. sulphuric acid (2 ml.), and after the mixture had been heated under reflux for 2 hours, the alcohol was distilled off, and the residue poured into water (*ca.* 200 ml.) and extracted with ether. The ethereal solutions were washed with dilute potassium carbonate solution and dried (Na_2SO_4). After filtration,

TABLE I.

Catalytic oxidation of elaidic acid at 120°.

Time (hours).	1	2	3	4	5	6	7	14	21
Iodine value (Wijs)	85·3	81·0	76·2	74·4	71·0	68·0	64·4	41·7	39·4
Acid value	197	185	178	175	170	166	161	149	137
Peroxide value (% active oxygen)	0·085	0·080	0·083	0·078	0·065	0·071	0·063	0·078	0·021
M (Rast)	—	—	—	—	—	—	—	—	549
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	—	—	—	Very slight

Catalytic oxidation of elaidic acid at 85°.

Time (hours).	2	4	6	10	14	18	23	29½	37
Iodine value (Wijs)	86·3	87·6	83·1	73·6	64·7	61·6	57·7	52·1	32·4
Acid value	196	196	196	192	185	184	181	176	176
Peroxide value	0·060	0·150	0·269	0·502	0·625	0·672	0·722	0·527	0·547
M (Rast)	—	—	—	—	—	—	—	—	332
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	—	—	—	Very slight

Catalytic oxidation of elaidic acid at 55°.

Time (hours).	1	2	3	4	5	6	7
Iodine value (Wijs)	88·4	87·7	87·3	87·5	88·4	87·9	86·4
Acid value	197	192	188	182	192	184	186
Peroxide value	0·014	0·016	0·016	0·020	0·022	0·024	0·027
M (Rast)	—	—	—	—	—	—	308
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	—	None

TABLE II.

Catalytic oxidation of methyl elaidate at 120°.

Time (hours).	6	12½	18½	24½
Iodine value (Wijs)	54·0	20·2	12·3	15·0
Sap. value	235	—	261	330
Peroxide value	0·805	0·575	0·394	0·276
M (Rast)	—	—	—	322
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	Rapid
$n_D^{20°}$	1·4563	1·4600	1·4621	1·4633

Catalytic oxidation of methyl elaidate at 85°.

Time (hours).	6	12	18	24	30	36	42	48
Iodine value (Wijs)	78·2	71·3	63·1	52·2	40·3	28·1	21·6	14·5
Sap. value	203	216	227	236	250	—	—	—
Peroxide value	0·42	0·93	1·38	1·63	1·71	1·66	1·52	1·33
M (Rast)	—	—	—	—	—	—	—	259
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	—	—	Slight
$n_D^{20°}$	1·4530	1·4548	—	—	—	1·4573	1·4569	1·4580

Catalytic oxidation of methyl elaidate at 55°.

Time (hours).	12	24	36	48	60	72
Iodine value (Wijs)	85·2	82·9	82·5	82·6	82·5	81·0
Sap. value	—	—	194	197	195	198
Peroxide value	0·065	0·089	0·130	0·159	0·256	0·334
M (Rast)	—	—	—	—	—	273
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	None
$n_D^{20°}$	—	—	—	—	—	1·4557

TABLE III.

Catalytic oxidation of n-*propyl elaidate at* 120°.

Time (hours).	3	6	12	18	24
Iodine value (Wijs)	73·5	54·2	—	36·3	28·6
Sap. value	187	209	240	275	292
Peroxide value	0·770	0·757	0·442	—	0·010
M (Rast)	—	—	—	—	375
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	Rapid
$n_D^{20°}$	1·4536	1·4547	1·4569	1·4629	1·4639

Catalytic oxidation of n-*propyl elaidate at* 85°.

Time (hours).	6	12	18	24	30	36	42	48
Iodine value (Wijs)	74·0	71·2	68·3	65·8	59·0	50·7	45·1	37·0
Sap. value	—	—	—	192	—	189	212	210
Peroxide value	0·214	0·478	0·801	1·04	1·39	1·50	1·59	1·59
M (Rast)	—	—	—	—	—	—	—	265
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	—	—	Very slight
$n_D^{20°}$	—	—	—	—	—	—	—	1·4563

Catalytic oxidation of n-*propyl elaidate at* 55°.

Time (hours).	12	24	36	48	60	70½
Iodine value (Wijs)	81·0	81·0	80·3	79·9	80·7	80·1
Sap. value	180	180	179	180	—	174
Peroxide value	0·074	0·133	0·156	0·201	0·224	0·268
M (Rast)	—	—	—	—	—	344
Reduction of Fehling's solution and ammoniacal silver nitrate	—	—	—	—	—	None

the ether was removed under reduced pressure. The product was then fractionated at 1—2 mm., in a Willstätter flask with a modified Perkin triangle: yield, 34·4 g.; m. p. 10·0°; b. p. 166—169°/1—2 mm.; n^{20} 1·4525 (Found: I V., 84·7. Calc. for $C_{19}H_{36}O_2$: I V., 85·8).

n-*Propyl Elaidate.*—50 G. of elaidic acid were treated with 200 ml. of *n*-propyl alcohol as above, and the final product fractionated: yield, 46·6 g.; b. p. 173—174°/1—2 mm.; n^{20} 1·4512 (Found: I V., 80·7. Calc. for $C_{20}H_{38}O_2$: I V., 81·7).

Barium Hydroxide Test.—Carbon dioxide evolved was absorbed in a saturated solution of barium hydroxide. At 55° only traces were detected with the esters and none with the acid. At 85° evolution occurred after 3—4 hours, whereas at 120° it occurred after only 15—30 minutes.

Increased-oxygen-rate Experiments.—These were initiated in order to observe (*a*) the effect of higher rates of oxygen flow on peroxide formation, and (*b*) the advantage or otherwise of a catalyst under these conditions. Three side-arm test-tubes (½ × 6 in.) were immersed in a boiling water-bath, each tube having a fine capillary (0·75-mm. bore) dipping into the reaction mixture (3·0 g. of elaidic acid with 0·05% of uranium or cobalt as catalyst; a control containing 3·0 g. of acid alone was included), through which a rapid stream of oxygen was passed. The results are tabulated in Table IV.

TABLE IV.

Catalytic oxidation of elaidic acid at 98°.

Time (hours).	2	4½	7
Peroxide value: (i) No catalyst	0·810	2·58	3·66
(ii) Uranium	1·37	2·99	3·99
(iii) Cobalt	0·231	0·361	0·670

One of us (M. N. T.) wishes to acknowledge a grant from the University of London Research Fund for assistance in this work.

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CHELSEA POLYTECHNIC, S.W.3.

[*Received, December 14th,* 1948.]

351. *Studies on Nitroamines. Part I. The Nitration of Some Methylenediamines.*

By F. Chapman.

The compounds (I), (IV; R = R′ = Me or $CH_2{\cdot}CH_2{\cdot}O{\cdot}NO_2$, and R = Me, R′ = Ph), and (V; n = 2, 3, or 4; R = morpholino or piperidino) have been prepared and nitrated with combinations of 98% nitric acid, acetic acid, acetic anhydride, and ammonium nitrate at various temperatures.

The research presented in this and the succeeding seven papers was carried out at the request of the Scientific Advisory Council of the Ministry of Supply during 1943—1945 under the direction of Professor R. D. Haworth, F.R.S. It was designed, in view of the increasing importance of nitroamines as explosives, to extend our knowledge of these compounds, and to throw light upon the reactions leading to the production of cyclonite (1 : 3 : 5-trinitrohexahydro-1 : 3 : 5-triazine) (III; n = 1) from hexamethylenetetramine.

The authors' thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish the results of their work.

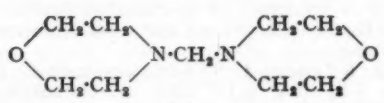
By nitration of substituted methylenediamines of the type $R_2N{\cdot}CH_2{\cdot}NR_2$, it was hoped that information might be obtained on the mode of nitrolysis of hexamethylenetetramine.

Addition of methylenebismorpholine (I) to 98% nitric acid at 0° gave morpholine nitrate in only 37% yield, calculated on the basis of one molecule of methylenebismorpholine yielding two molecules of morpholine nitrate. This suggested that nitrolysis gave equimolecular amounts of morpholine nitrate and morpholinomethyl nitrate (II; R = $CH_2{\cdot}O{\cdot}NO_2$). This view was supported by the absence of formaldehyde from the nitration liquors, but morpholinomethyl nitrate could not be isolated.

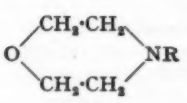
Morpholine nitrate was also formed on treatment of (I) with 98% nitric acid and acetic anhydride at 0°; when the reaction mixture was warmed to 40° or when nitration was effected with a mixture of 98% nitric acid, acetic acid, and acetic anhydride at 55°, 4-nitrosomorpholine (II; R = NO) was obtained. Presumably the nitrosation is due to the reduction of nitric acid by formaldehyde liberated during the reaction.

Nitration of (I) with 98% nitric acid, acetic anhydride, and ammonium nitrate at 55° resulted in the formation of cyclonite (III; n = 1) and 4-nitromorpholine (II; R = NO_2) (Chute *et al.*, *Canadian J. Res.*, 1948, **26**, *B*, 114). Cyclonite probably arises from the combination of the liberated formaldehyde with ammonium nitrate, followed by nitration. This reaction is facilitated by the presence of ammonium nitrate, since the small yields obtained by Chute *et al.* (*loc. cit.*) without a (chloride) catalyst were increased to 47% by the addition of this salt.

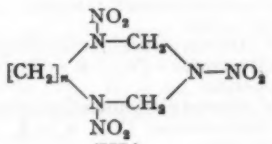
The nitration of tetramethylmethylenediamine (IV; R = R′ = Me) (Henry, *Bull. Acad. roy. Belg.*, 1893, [iii], **26**, 203) with 98% nitric acid in presence of acetic anhydride and ammonium nitrate at 55° or 75° yielded dimethylnitroamine and cyclonite, and threw little light on nitrolysis.



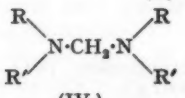
(I.)



(II.)



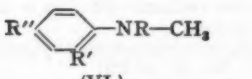
(III.)



(IV.)

$CH_2R{\cdot}N(NO_2){\cdot}[CH_2]_n{\cdot}N(NO_2){\cdot}CH_2R$

(V.)



(VI.)

Bismorpholinomethylethylenedinitroamine (V; n = 2; R = morpholino) (see Part III), nitrated with 98% nitric acid, acetic anhydride, and ammonium nitrate at 55—60°, yielded 1 : 3 : 5-trinitro-1 : 3 : 5-triaza*cyclo*heptane (III; n = 2; Myers and Wright, "Nitrolysis of Hexamine, Part IV," *Canadian J. Res.*, in the press) and 4-nitromorpholine. 1 : 3 : 5-Trinitro-1 : 3 : 5-triaza*cyclo*heptane and 1-nitropiperidine were prepared similarly from bispiperidinomethylethylenedinitroamine (Franchimont, *Rec. Trav. chim.*, 1910, **29**, 306). In the same way, nitration of bismorpholinomethyltrimethylenedinitroamine (V; n = 3; R = morpholino)

(see Part III) gave 4-nitromorpholine and 1 : 3 : 5-*trinitro*-1 : 3 : 5-*triaza*cyclo*octane* (III; $n = 3$). On the other hand, nitration of bismorpholinomethyltetramethylenedinitroamine (V; $n = 4$; R = morpholino) (see Part III) gave 4-nitromorpholine and a water-insoluble oil which could not be identified.

Nitration of methylenebismonomethylaniline (IV; R = Me, R′ = Ph) (von Braun, *Ber.*, 1908, **41**, 2147) at 0°, with a mixture of 98% nitric acid and acetic anhydride, gave a small yield of 2 : 4 : *N*-trinitro-*N*-methylaniline (VI; R = R′ = R″ = NO_2), but at 55°, with a mixture of 98% nitric acid, acetic anhydride, and ammonium nitrate, *p* : *N*-dinitro-*N*-methylaniline (VI; R = R″ = NO_2, R′ = H) was produced.

Methylenebis-(2 : 2′-*dinitroxydiethylamine*) (IV; R = R′ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}NO_2$) proved quite stable to nitration and was recovered after treatment with 98% nitric acid at 25° or 45° or with a mixture of 98% nitric acid, acetic anhydride, and acetic acid at 55°.

Experimental.

Methylenebismorpholine (I).—Morpholine (17·7 c.c.) was added gradually, with cooling, to a slight excess of 40% formaldehyde (8 c.c.). After the mixture had been heated on a water-bath for 0·5 hour, anhydrous potassium carbonate was added until the water was absorbed. The resultant oil was decanted and distilled. *Methylenebismorpholine* (16·2 g.) was obtained as a colourless liquid, b. p. 110—112°/10 mm. (Found: equiv., by titration with 0·1N-hydrochloric acid, 95·2, 94·8, 94·8. $C_9H_{18}O_2N_2$ requires equiv., 93).

Methylenebis-(2 : 2′-*dinitroxydiethylamine*) (IV; R = R′ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}NO_2$).—Dinitroxydiethylamine nitrate (2·6 g.) prepared by the method of Chute *et al.* (*Canad. J. Res.*, 1948, **26**, *B*, 89) was dissolved in hot water (10 c.c.), and dilute sodium hydroxide solution added until the mixture was alkaline to phenolphthalein. The dinitroxydiethylamine so liberated was extracted with ether (2 × 10 c.c.), the ether extract dried, and the ether removed. The impure residue (1·7 g.) (which showed a tendency to explode if heated alone on a water-bath) was treated with 40% formaldehyde solution (1·0 c.c.), and the mixture heated on the water-bath for 2 hours. The solution so obtained was cooled, and *methylenebis*-(2 : 2′-*dinitroxydiethylamine*) precipitated by addition of methyl alcohol, collected, and dried. After recrystallisation from aqueous methyl alcohol it was obtained as minute prisms (0·6 g.), m. p. 170° (decomp.) (Found: C, 27·3; H, 4·8; N, 20·0. $C_5H_{10}O_{10}N_4$ requires C, 26·9; H, 4·5; N, 20·9%).

Nitration of the Methylenediamines.—In general this was carried out under some or all of the following conditions.

(a) *Using 98% nitric acid at 0°.* The methylenediamine (1 g.) was added slowly to well-stirred 98% nitric acid (1·6 g.) at 0°.

(b) *Using 98% nitric acid, acetic anhydride, and acetic acid at 50—60°.* The methylenediamine (1 g.) was added slowly and simultaneously with a mixture of 98% nitric acid (1·3 g.) and acetic anhydride (2·4 g.) to a mixture of acetic acid (2·5 g.) and acetic anhydride (2·4 g.) at the appropriate temperature.

(c) *Using 98% nitric acid, acetic anhydride, and ammonium nitrate at 50—60°.* The methylenediamine (1 g.) was added slowly and simultaneously with a solution of ammonium nitrate (1·6 g.) in 98% nitric acid (2·2 g.) to acetic anhydride (8 g.) at 50—60°.

Compounds obtained from 1 G. of the Methylenediamine.

Compound nitrated.	Conditions.	Product(s).
Methylenebismorpholine (I)	*a*	Morpholine nitrate (0·4 g.).
	b	4-Nitrosomorpholine (0·3 g.).
	c	4-Nitromorpholine (II; R = NO_2) (0·2 g.) and cyclonite (0·04 g.).
Methylenebisdimethylamine (IV; R = R′ = Me)	*c*	Dimethylnitroamine (0·2 g.) and cyclonite (0·05 g.).
NN′-Bismorpholinomethylethylenedinitroamine (V; $n = 2$; R = morpholino)	*c*	1 : 3 : 5-Trinitro-1 : 3 : 5-triaza*cyclo*heptane (III; $n = 2$) (0·5 g.) and 4-nitromorpholine (0·2 g.).
NN′-Bismorpholinomethyltrimethylenedinitroamine (V; $n = 3$, R = morpholino)	*c*	1 : 3 : 5-*Trinitro*-1 : 3 : 5-*triaza*cyclo*octane* (III; $n = 3$) (0·4 g.) * and 4-nitromorpholine (0·3 g.).
NN′-Bismorpholinomethyltetramethylenedinitroamine (V; $n = 4$, R = morpholino)	*c*	4-Nitromorpholine (<0·1 g.).
Methylenebismonomethylaniline (IV; R = Me, R′ = Ph)	*b*	2 : 4 : *N*-Trinitro-*N*-methylaniline (VI; R = R′ = R″ = NO_2) (0·07 g.).
	c	*p* : *N*-Dinitro-*N*-methylaniline (VI; R = R″ = NO_2, R′ = H) (0·15 g.).
Methylenebis-(2 : 2′-dinitroxydiethylamine) (IV; R = R′ = $CH_2{\cdot}CH_2{\cdot}O{\cdot}NO_2$)	*a* and *b*	Initial material (0·8 g.) recovered by addition to ether.

Except for (III; $n = 3$), compounds were identified by mixed melting point with authentic specimens.
* M. p. 164—166° (Found: C, 24·0; H, 3·9; N, 33·8. $C_6H_{10}O_8N_6$ requires C, 24·0, H, 4·0; N, 33·6%).

In all cases the solids which separated during the addition of the methylenediamine were collected, washed, generally with cold acetone, and crystallised. The nitration mixture was then diluted with

a large volume of water, and any solids which then separated were collected, washed, dried, and crystallised. The dilute nitration liquors were then extracted with chloroform (10 × 10 c.c.), the chloroform extract was washed with saturated sodium hydrogen carbonate solution, dried, and evaporated. The residue was distilled at 0·5 mm. and crystallised.

This procedure was not adopted with compound (IV; R = R′ = CH_2·CH_2·O·NO_2). After nitration of this under conditions (*a*) or (*b*), the nitration mixture was cooled if necessary, and then added slowly with stirring to ice-cold ether.

Results are detailed in the annexed Table.

Conversion of Morpholine Nitrate into 4-*Nitromorpholine* (II; R = NO_2) *by Acetic Anhydride and Ammonium Nitrate.*—98% Nitric acid (1 c.c.) was added slowly, with stirring, to acetic anhydride (43·4 g.); ammonium nitrate (13 g.), followed by morpholine nitrate (6·4 g.), were then added, the temperature was raised to 65° during 0·5 hour, and maintained at 65° for a further 1 hour. After cooling, the solution was poured on crushed ice (200 g.) and extracted with chloroform (10 × 10 c.c.). The extract was washed with saturated sodium hydrogen carbonate solution and dried, and the solvent removed. The oily residue was distilled, to give 4-nitromorpholine (2·9 g.), b. p. 84°/1·3 mm., which crystallised from alcohol in prisms (2·7 g.), m. p. 52—53°.

Identification of p : N-*Dinitro*-N-*methylaniline* (VII; R = R″ = NO_2, R′ = H) *by Conversion into* 2 : 4-*Dinitro*-N-*methylaniline* (VII; R = H, R′ = R″ = NO_2).—*p* : *N*-Dinitro-*N*-methylaniline (0·1 g.) was added gradually to concentrated sulphuric acid (1 c.c.) and set aside overnight. The solution was poured into water (10 c.c.) and made slightly alkaline. The bright-yellow precipitate of 2 : 4-dinitro-*N*-methylaniline was collected and crystallised from aqueous acetone as aggregates of minute prisms (0·07 g.), m. p. 175°, undepressed by admixture with an authentic specimen.

THE UNIVERSITY, SHEFFIELD, 10. [*Received, December 15th*, 1948.]

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352. *Studies on Nitroamines. Part II. The Nitration of Some Methylenebisamides and Related Compounds.*

By R. C. BRIAN and ALEX. H. LAMBERTON.

Methylenebisacetamide (II; R = Ac, R′ = H) is convertible, by nitration and subsequent hydrolysis, into methylenedinitroamine (I; R = H). Attempts to prepare methylenedinitroamine and its analogues from related bisamides were unsuccessful.

IN the course of work undertaken primarily to provide a synthetical proof of the structure of methylenedinitroamine (I; R = H), first isolated, as a degradation product during nitrolysis of hexamine, by workers at Bristol University (private communication), the nitration of a number of bisamides (II; R = Ac, R′ = H, Me, or Ph; R = CO_2Et, R′ = H or Me; or R = CO·NMe_2, R′ = H) and (III) has been studied. Cleavage of the N–C–N linkages

NO_2·NR·CH_2·NR·NO_2 (I.) NHR·CHR′·NHR (II.) CO_2Et·NMe·CH_2·NMe·CO_2Et (III.)

appeared to be the commonest reaction, but methylenebis-*N*-acetamide (II; R = Ac, R′ = H) was unaffected by cold 98% nitric acid. Whilst addition of ether precipitated the *mononitrate*, introduction of acetic anhydride led to the formation of *methylenebis*-N-(N-*nitro*)-*acetamide* (I; R = Ac), from which methylenedinitroamine could best be obtained by hydrolysis with aqueous ammonia.

Ethylidenebis-*N*-acetamide (II; R = Ac, R′ = Me) was less stable to 98% nitric acid than was the methylene homologue, and no isolable nitration product could be obtained from nitric acid–acetic anhydride mixtures. Benzylidenebis-*N*-acetamide (II; R = Ac, R′ = Ph) yielded nitrobenzylidenebisacetamides with 98% nitric acid, the *p*-isomer being predominant. Introduction of acetic anhydride caused cleavage to *p*-nitrobenzaldehyde.

Nitric acid treatment of methylenebis-*N*-urethane (II; R = CO_2Et, R′ = H) yielded nitrourethane and an (unidentified) neutral oil. Similar treatment of ethylidenebis-*N*-urethane (II; R = CO_2Et, R′ = Me) gave a greater yield of nitrourethane, but no neutral product. The stability of nitrourethane in nitric acid was greater than anticipated (Thiele and Lachmann, *Ber.*, 1894, **27**, 1519; *Annalen*, 1895, **288**, 269), and a direct preparation from urethane and nitric acid compares favourably with the traditional method. *Methylenebis*-(N-*methylurethane*) (III) yielded methylnitroamine on treatment with 98% nitric acid and subsequent ammonolysis, whilst from methylenebis-(*as*-dimethylurea) (II; R = CO·NMe_2, R′ = H) there was obtained a small yield of dimethylnitroamine.

EXPERIMENTAL.

Methylenebis-N-*acetamide* (II; R = Ac, R′ = H).—Acetamide (118 g.) and paraformaldehyde (33 g.) were heated in an oil-bath at 155° for 17 hours with, and for a further 90 minutes without, a

reflux condenser. The melt yielded methylenebis-*N*-acetamide (60 g.), m. p. 196°, on crystallisation from alcohol (350 ml.); a further 20 g. were obtainable by evaporation and reheating of the residue. This method, based on G.P. 164611 (*Centr.*, 1905, II, 1751), appeared more convenient than that of Noyes and Forman (*J. Amer. Chem. Soc.*, 1933, **55**, 3493).

Methylenebis-N-acetamide Mononitrate.—Methylenebisacetamide (5 g.) was added at −5° to 98% nitric acid (30 ml.); the product appeared to be stable since, after 25 minutes at < +5° and precipitation at about −30° by the dropwise addition of precooled ether (105 ml.), the *nitrate* (6·4 g., after washing with ether) could be collected. This crystallised from chloroform in needles, m. p. 102° (Found: equiv., 195. $C_5H_{10}O_4N_2,HNO_3$ requires equiv., 193), which gave potassium nitrate and methylenebis-*N*-acetamide on neutralisation with alcoholic potassium hydroxide.

Methylenebis-N-(N-nitro)acetamide (I; R = Ac).—98% Nitric acid (190 ml.) was added at 15° during 30 minutes to a vigorously stirred suspension of methylenebis-*N*-acetamide (65 g.) in acetic anhydride (190 ml.). After 3 hours at 15°, the solution was run in a stream during 30 minutes into vigorously stirred ice (800 g.) and ice-water (1 l.). The crude nitration product was collected and washed with water; it could be hydrolysed to methylenedinitroamine without further treatment but, if it was dried in a vacuum, the yield was 60 g. *Methylenebis-N-(N-nitro)acetamide* separated from aqueous methyl alcohol in rhombic plates, m. p. 63°, insoluble in water or ligroin, sparingly soluble in ether, moderately soluble in alcohol, and easily soluble in ethyl acetate, acetone, benzene, chloroform, nitromethane, or dioxan (Found: C, 27·3; H, 3·9; N, 25·7. $C_6H_8O_6N_4$ requires C, 27·2; H, 3·6; N, 25·4%). The solid was reasonably stable after crystallisation, and has been kept on porcelain in a desiccator for 3 years, though extensive decomposition occurred in 3 months on storage in glass screw-capped bottles.

The yield was unaffected by the order of addition of the components, and the quantities of both acid and anhydride could be doubled without decrease of yield, but a decrease in the quantity of acid was markedly deleterious. None of the product was obtained in 98% nitric acid alone, or in a 98% nitric acid–fuming sulphuric acid mixture. At 0° the yield rose smoothly to about 40% after 3½ hours and thereafter fell very slowly; at higher temperatures greater maxima were obtained more rapidly (*e.g.*, 55% after 2½ hours at 16°), but the subsequent fall in yield was also accelerated, and only an oil (presumably tetranitromethane) was produced at 30°.

Methylenedinitroamine (I; R = H).—The precipitate of crude methylenebis-*N*-(*N*-nitro)acetamide (from 65 g. of methylenebisacetamide) was collected, washed with water, drained, and added portionwise to well-stirred aqueous ammonia [100 ml. (*d* 0·88) in 140 ml. of water] kept at <20°. After a further 5 minutes, barium chloride solution (85 g. of the dihydrate in 250 ml. of water) was run in, and the suspension cooled in about 20 minutes to 4°. The barium salt of methylenedinitroamine was collected, washed with cold 50% aqueous acetone, and added to hydrochloric acid (60 ml. of conc. acid in 140 ml. of water); after the solution had been filtered, extracted with ether (7 × 100 ml.), dried (Na_2SO_4), and concentrated to about 60 ml., methylenedinitroamine (28 g.), m. p. 98—101° (decomp.), was precipitated by the addition of two volumes of warm ligroin (b. p. 40—60°). This method, based on a knowledge of the stability of methylenedinitroamine in aqueous solutions at certain pH values (Part VII of this series) produced methylenedinitroamine (in 25% w/w yield, calc. on acetamide) of quality satisfactory for preparative work; pure material was obtained by one crystallisation from ether. Owing to decomposition, the m. p. is less satisfactory as a criterion of purity than is titration with alkali hydroxide to thymolphthalein.

Attempted Nitration of Ethylidenebis-N-acetamide (II; R = Ac, R′ = Me).—(*a*) Ethylidenebis-*N*-acetamide (1·5 g.) (Noyes and Forman, *loc. cit.*) was added to 98% nitric acid (9 ml.) in 6 minutes at −5°. After a further 9 minutes at −5°, the mixture was run dropwise into cooled ether; on decantation and stirring in fresh ether the resultant oil solidified (0·47 g.) and yielded ethylidenebis-*N*-acetamide (0·22 g.), m. p. 177°, on crystallisation from alcohol. No insoluble material was obtained by running a similar nitration mixture into water.

(*b*) Ethylidenebis-*N*-acetamide in acetic anhydride was treated with nitric acid as detailed for the methylene homologue; no water-insoluble solid was obtained at any time during ageing at 15°, 0°, or −10°.

Nitration of Benzylidenebis-N-acetamide (II; R = Ac, R′ = Ph).—(*a*) Benzylidenebis-*N*-acetamide (2 g.) (Noyes and Forman, *loc. cit.*) was added to 98% nitric acid (10 ml.) in 10 minutes at 0°. After 20 minutes at 1°, the mixture was run dropwise into cooled ether (60 ml.). The mixture of nitrobenzylidenebis-*N*-acetamides (1·83 g.), m. p. 230—240°, was collected and twice crystallised from alcohol–water (9 : 1); needles, m. p. 260—262° (Found: C, 52·7; H, 5·3. Calc. for $C_{11}H_{13}O_4N_3$: C, 52·6; H, 5·2%), were obtained (Ittyerah and Pandya, *Proc. Indian Acad. Sci.*, 1942, **15**, *A*, 258, give 272° as the m. p. of the pure *p*-isomer). 0·25 G. of the crude nitrobenzylidenebis-*N*-acetamides, m. p. 230—240°, was heated at 90° for 4 hours with 2N-hydrochloric acid (20 ml.). Insoluble material was collected; *p*-nitrobenzaldehyde (0·06 g.), m. p. 99—102°, separated on cooling.

(*b*) Benzylidenebis-*N*-acetamide (5 g.) was suspended in acetic anhydride (20 ml.) and 98% nitric acid (21 ml.) added at 15° in 15 minutes. After 3 hours at 15° the mixture was poured into ice and water (250 ml.). The resultant oil was extracted with ether, washed with sodium bicarbonate, dried, and evaporated; after 3 weeks, *p*-nitrobenzaldehyde (0·8 g.), m. p. 95—98°, was deposited. A similar experiment showed that nitrobenzylidenebis-*N*-acetamides, isolable at first by running into ether, were destroyed during the ageing process.

Nitration of Methylene- and Ethylidene-bisurethanes (II; R = CO_2Et, R′ = H or Me).—(*a*) Methylenebisurethane (5 g.) (better prepared by condensation in 0·1N-hydrochloric acid in place of the 0·02N-acid of Conrad and Hock, *Ber.*, 1903, **36**, 2206) was added in 7 minutes to 98% nitric acid (25 ml.) at −8°. After a further 8 minutes at −8°, the mixture was poured on ice (300 g.) and extracted with ether, and the extract washed with sodium hydrogen carbonate solution. The residual ether yielded on evaporation a neutral oil (1·7 g.) from which no crystalline material could be isolated. The carbonate washings were acidified and extracted with ether; passage of gaseous ammonia through the extract precipitated ammonium nitrourethane (1·74 g.), m. p. 162° (decomp.), identified by comparison with an authentic sample and by conversion into the free acid.

(*b*) Ethylidenebisurethane (5 g.) [prepared in 78% yield by treatment of urethane (17·8 g.) in water (55 ml.) with acetaldehyde (4·6 g.) and concentrated hydrochloric acid (1 ml.); cf. Nencki, *Ber.*, 1874, **7**, 160] yielded, on similar treatment, no neutral oil, and an enhanced yield (5·6 g.) of ammonium nitrourethane, m. p. 160° (decomp.).

Direct Nitration of Urethane.—Urethane (4·45 g.) was added to 98% nitric acid (21 ml.) in 10 minutes at −5°. After 25 minutes at 0° the mixture, worked up as described for the nitration of methylenebisurethane, yielded ammonium nitrourethane (7·26 g.), m. p. 156°; crystallisation from methylated spirits (40 ml.) yielded the pure salt (4·7 g.), m. p. 171°.

Methylenebis-(N-methylurethane) (III).—*N*-Methylurethane (30 ml.) was shaken for 36 hours with 0·1N-hydrochloric acid (180 ml.) and 38% formaldehyde (14 ml.). The oil which separated was extracted with ether, dried, and distilled. The *product* (III) (16 g.) had b. p. 136—142°/14 mm. (Found: C, 49·8; H, 8·4. $C_9H_{18}O_4N_2$ requires C, 49·5; H, 8·3%). By nitration and working up as described for methylenebisurethane, a neutral oil was obtained; passage of ammonia through an ethereal solution of this product precipitated the hygroscopic ammonium methylnitroamine as a yellow oil. Excess of anhydrous sodium sulphate was added, the solid collected, decomposed with acid, and extracted with ether. Methylnitroamine (1·1 g.), m. p. 37°, not depressed by an authentic specimen, was obtained on evaporation.

Nitration of Methylenebis-(as-dimethylurea) (II; R = $CO{\cdot}NMe_2$, R′ = H).—Methylenebis-(*as*-dimethylurea) (2 g.) (Einhorn, *Annalen*, 1908, **361**, 136; for dimethylurea, see Davis and Blanchard, *J. Amer. Chem. Soc.*, 1929, **51**, 1798) was added to 98% nitric acid (10 ml.) in 15 minutes at −8°. Slight evolution of gas occurred during dissolution, and after a further 4 minutes at −8° the mixture was poured on ice (100 g.). Extraction with ether yielded only dimethylnitroamine (0·2 g.), m. p. 53—56°, identified by comparison with an authentic sample.

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353. *Studies on Nitroamines. Part III. The Reactions of Nitroamines with Formaldehyde.*

By DAVID WOODCOCK.

The *N*-hydroxymethyl compounds (I; R = $CH_2{\cdot}OH$; n = 1, 3, or 4) and (II; R = $CH_2{\cdot}OH$) have been prepared by utilising the reversible reaction between the corresponding nitroamine and formaldehyde. Characteristic derivatives (I; R = morpholinomethyl; n = 3 or 4) and (II; R = morpholinomethyl) are formed with morpholine.

FRANCHIMONT (*Rec. Trav. chim.*, 1910, **29**, 306) showed that nitroamines reacted with formaldehyde in the presence of piperidine or piperazine, but stated that no reaction took place in the absence of bases (*ibid.*, 1895, **14**, 48; 1910, **29**, 306). It has now been found that ethylenedinitroamine (I; n = 2; R = H) condenses with 40% formaldehyde to form *N-hydroxymethylethylenedinitroamine* (II; R = $CH_2{\cdot}OH$), m. p. 127—129° (decomp.), but attempts to obtain the *NN′*-bishydroxymethyl derivative (I; n = 2; R = $CH_2{\cdot}OH$) were unsuccessful even in the presence of a large excess of formaldehyde.

(I.) $NO_2{\cdot}NR{\cdot}[CH_2]_n{\cdot}NR{\cdot}NO_2$ $NO_2{\cdot}NR{\cdot}CH_2{\cdot}CH_2{\cdot}NH{\cdot}NO_2$ (II.)

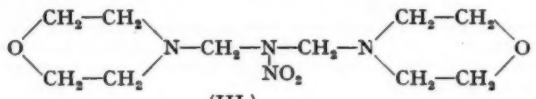
The product (II; R = $CH_2{\cdot}OH$) reacts with morpholine to give a crystalline *derivative* (II; R = morpholinomethyl), but with an excess of the base in the presence of an excess of formaldehyde gives the *compound* (I; n = 2; R = morpholinomethyl). These morpholine derivatives are highly characteristic and can be accurately titrated with 0·1N-sodium hydroxide using thymolphthalein as indicator, and the formaldehyde liberated (see Part V) can be conveniently determined.

Treatment of ethylenedinitroamine with excess of piperidine in the presence of excess of 40% formaldehyde gave the compound (I; n = 2; R = piperidinomethyl), m. p. 112—114° (decomp.) (cf. Franchimont, *Rec. Trav. chim.*, 1910, **29**, 207), but diethylamine or monomethylaniline failed to give crystalline products.

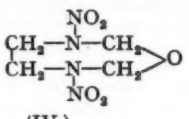
Methylenedinitroamine (I; n = 1; R = H), when treated in ethyl acetate solution with dry gaseous formaldehyde, formed the *compound* (I; n = 1; R = $CH_2{\cdot}OH$), but the yield was poor and erratic under a variety of conditions. Confirmation of the structure of this product was obtained by its conversion by acetyl chloride into *NN′*-bisacetoxymethylmethylene dinitroamine (I; n = 1; R = $CH_2{\cdot}OAc$) (first prepared by Roberts, private communication).

Treatment of the diol (I; n = 1; R = $CH_2{\cdot}OH$) with excess of morpholine failed to give the dimorpholino-compound; the water-soluble product evolved formaldehyde readily on warming the solution, and gave elementary analysis results in good agreement with those required for *substance* (III). Its formation is apparently made possible by a breakdown of the

nitroamine into nitroamine in the presence of morpholine and formaldehyde, and the structure was confirmed by synthesis from these components.



(III.)



(IV.)

Trimethylenedinitroamine (I; $n = 3$; R = H) and tetramethylenedinitroamine (I; $n = 4$; R = H) condensed with warm 40% aqueous formaldehyde to give the *diols* (I; R = CH_2·OH; $n = 3$ or 4, respectively), which with excess of morpholine yielded characteristic derivatives (I; R = morpholinomethyl, $n = 3$ or 4, respectively); both these and the diols were accurately titratable using 0·1N-sodium hydroxide and thymolphthalein.

During experiments with *N*-hydroxymethylethylenedinitroamine (II; R = CH_2·OH) it was found that heating at 130—140° gave an alkali-insoluble substance, m. p. 154—155°, in 15% yield. Analytical figures suggested that this was 3 : 6-*dinitro*-1-*oxa*-3 : 6-*diaza*cyclo*heptane* (IV), and 98% nitric acid at −20° gave the ether-insoluble NN′-*bisnitroxymethylethylenedinitroamine* (I; $n = 2$; R = CH_2·O·NO_2) which was converted into the *dimethoxy-* (I; $n = 2$; R = CH_2·OMe) and diacetoxy-compound (I; $n = 2$; R = CH_2·OAc) (Myers and Wright, "Nitrolysis of Hexamine, Part IV," *Canadian J. Res.*, in the press) by methyl alcohol and acetic acid, respectively.

Attempts to synthesise (IV) by treating ethylenedinitroamine and excess of 40% aqueous formaldehyde with acetic anhydride at 65° were unsuccessful; (II; R = CH_2·OH) was usually obtained, but in one experiment a small amount of *NN′*-bisacetylethylenedinitroamine (I; $n = 2$; R = COMe) was isolated by evaporation of the solution in a vacuum below 70°. In view of statements in the literature (Franchimont and Klobbie, *Rec. Trav. chim.*, 1889, **8**, 297; 1910, **29**, 303; Thomas, *ibid.*, 1890, **9**, 85) that smooth acylation of primary nitroamines has seldom been realised, it is of interest to record that *NN′*-bisacetylethylenedinitroamine can be prepared by stirring ethylenedinitroamine with acetic anhydride at 65°; at higher temperatures no solid product could be isolated.

Experimental.

N-*Hydroxymethylethylenedinitroamine* (II; R = CH_2·OH).—40% Aqueous formaldehyde (20 c.c.; 2 mols.) was added to ethylenedinitroamine (20 g.) in boiling water (110 c.c.). The *product* (22·1 g.) which crystallised from the cooled mixture in stout prisms, m. p. 127—130° (decomp.), was collected and dried (Found : C, 20·3, 20·4; H, 4·5, 4·0; N, 31·1, 31·4; CH_2O, 16·4; equiv., 89·8, 90·8. $C_3H_8O_5N_4$ requires C, 20·0; H, 4·4; N, 31·1; CH_2O, 16·6%; equiv., 90). Prolonged heating on a water-bath caused decomposition with loss of formaldehyde, and attempted recrystallisation from hot methyl, ethyl, or butyl alcohol yielded only ethylenedinitroamine. The product was insoluble in cold ether, chloroform, and methyl or ethyl alcohol, moderately soluble in acetone, and readily soluble in warm acetic anhydride.

N-*Morpholinomethylethylenedinitroamine* (II; R = morpholinomethyl).—*N*-Hydroxymethylethylenedinitroamine (0·45 g.) was dissolved in a warm mixture of morpholine (0·22 c.c.; 1 mol.) and ethyl acetate (3 c.c.); the *product* obtained after cooling crystallised in rosettes of monoclinic prisms (0·52 g.), m. p. 128—130° (decomp.) (Found : C, 34·0; H, 6·0; CH_2O, 12·2; equiv., 124·0. $C_{17}H_{18}O_5N_5$ requires C, 33·7; H, 6·0; CH_2O, 12·0%; equiv., 124·5).

NN′-*Bismorpholinomethylethylenedinitroamine* (I; $n = 2$; R = morpholinomethyl).—Ethylenedinitroamine, *N*-hydroxymethylethylenedinitroamine, or the monomorpholinqmethyl compound described above, was warmed with excess of 40% formaldehyde and morpholine in ethyl acetate for 5 minutes; on cooling the mixture, the *product* separated in a mass of felted needles, m. p. 170—171° (reddening and decomp.) (Found : C, 41·0; H, 6·8; CH_2O, 19·7; equiv., 174·0. $C_{12}H_{24}O_6N_6$ requires C, 41·3; H, 6·9; CH_2O, 17·2%; equiv., 174·0).

NN′-*Bishydroxymethylmethylenedinitroamine* (I; $n = 1$; R = CH_2·OH).—Gaseous formaldehyde was passed to saturation through a solution of methylenedinitroamine (0·5 g.) in ethyl acetate (1 c.c.); the mixture was cooled to 0°, and the *product*, rosettes of prisms (0·53 g.), m. p. 70—75° (decomp.), collected (Found : C, 19·3; H, 4·1; N, 27·8; equiv., 98·1. $C_3H_8O_6N_4$ requires C, 18·4; H, 4·1; N, 28·6%; equiv., 98·0). The yields from three similar experiments were 0·39, 0·34, 0·30 g., respectively; however, on many occasions the product failed to crystallise, and the solution was then evaporated in vacuo and acetylated, as described below, to give the diacetate.

NN′-*Bisacetoxymethylmethylenedinitroamine* (I; $n = 1$; R = CH_2·OAc).—This was first prepared by Roberts (private communication). The above-mentioned diol (0·2 g.) was heated under reflux with acetyl chloride (1 c.c.) for 5 minutes; after removal of excess of solvent in a vacuum, the residue was triturated with water (5 c.c.), and the solid (0·26 g.) was collected and dried in a vacuum; recrystallisation from methyl alcohol gave prisms (0·18 g.), m. p. 103—104° (Found: C, 30·4; H, 4·6; N, 19·9; CH_2O, 31·6. Calc. for $C_7H_{12}O_6N_4$: C, 30·0; H, 4·3; N, 20·0; CH_2O, 32·1%). Attempted acetylation of the diol with acetic anhydride at 70° yielded methylenedinitroamine, and at 140° an amorphous product; no reaction occurred at room temperature.

Bismorpholinomethylnitroamine (III).—(*a*) A solution of methylenedinitroamine (0·54 g.) in 40%

aqueous formaldehyde (0·60 c.c.) was cooled below 0°, and morpholine (0·70 c.c., 2 mols.) added with stirring. After half an hour, the white *solid* (1·05 g.), m. p. 95—97° (decomp.), was collected and dried (Found: C, 45·7; H, 7·7; N, 20·6; CH_2O, 24·6, 24·2, 23·7; *M* (cryoscopic in benzene), 210. $C_{10}H_{20}O_4N_4$ requires C, 46·1; H, 7·7; N, 21·5; CH_2O, 23·0%; *M*, 260). (*b*) A solution of methylenedinitroamine (0·5 g.) in ethyl acetate (0·8 c.c.) was saturated with gaseous formaldehyde at 0°, and morpholine (0·7 c.c., 2 mols.) added with shaking. After standing overnight at 0°, the solid (0·82 g.), m. p. 96—100° (decomp.), undepressed by admixture with the product from (*a*), was collected, washed with ether, and dried in a vacuum. (*c*) A solution of nitroamide (0·15 g.) in 40% aqueous formaldehyde (0·6 c.c., 3 mols.) was cooled to −10°, and morpholine (0·44 c.c., 2 mols.) added with stirring. After being kept at 0° for 3 hours, during which some effervescence was observed, the white solid (0·15 g.), m. p. 98—100° (decomp.), undepressed by admixture with products from (*a*) or (*b*), was collected, washed with ether, and dried.

NN′-*Biscarbomethoxytrimethylenediamine*.—The instructions of Ing and Manske (*J.*, 1926, 2349) led to a low yield of poor quality αγ-diphthalimidopropane. The following modification was therefore adopted. Finely powdered potassium phthalimide (Hale and Britton, *J. Amer. Chem. Soc.*, 1919, **41**, 844) (96·5 g.) and trimethylene dibromide (30 c.c., 0·6 mol.) were heated in an oil-bath at 180—200° for 2·5 hours, and any unchanged bromide was removed in steam. The residual sticky mass, crystallised from glacial acetic acid, yielded αγ-diphthalimidopropane (64·2 g.), m. p. 194—196°.

This product (25 g.) was heated in boiling 95% ethyl alcohol (70 c.c.) with hydrazine hydrate (100%; 7·5 c.c.); a clear solution was obtained after 5 minutes, but in a further 10 minutes a gelatinous precipitate had formed. After cooling, excess of 2N-hydrochloric acid was added, and the phthalhydrazide collected; the filtrate was concentrated and cooled, and a further amount of hydrazide collected. The aqueous solution of trimethylenediamine dihydrochloride was cooled to <0° and stirred during the addition of sodium hydroxide (6·25 g., 2 mols.) in water (10 c.c.), and again during the simultaneous dropwise addition of methyl chloroformate (11·5 c.c., 2 mols.) and sodium hydroxide (6·25 g., 2 mols.) in water (10 c.c.). After the mixture had been stirred for a further 0·5 hour, the product was extracted with chloroform, dried, and freed from solvent, and the residue distilled. The distillate (7·3 g.), b. p. 154—156°/0·4 mm., solidified on cooling and crystallised from ether–light petroleum (b. p. 40—60°) in plates, m. p. 63—68°. Franchimont and Klobbie (*Rec. Trav. chim.*, 1888, **7**, 747) give m. p. 74—75°.

Trimethylenedinitroamine (I; $n = 3$; R = H).—The foregoing compound was converted into the *NN′*-dinitro-derivative (I; $n = 3$; R = CO_2Me), m. p. 89—90°, by the method of Franchimont and Klobbie (*loc. cit.*), but their method of hydrolysis was modified as follows. The dinitro-compound (4 g.) was warmed with aqueous ammonia (8 c.c., *d* 0·88) for 5 minutes, water (5 c.c.) added, and excess of ammonia removed by evaporation at 100°. The oily residue was cooled, acidified with 2N-hydrochloric acid (Congo-red), extracted with ethyl acetate, dried, and freed from solvent. The solid residue (m. p. 58—64°) crystallised from water or ether–light petroleum (b. p. 40—60°) in stout prisms (1·46 g.), m. p. 68—69°. The mother-liquors gave a further amount (0·56 g.), m. p. 63—68°. Franchimont and Klobbie give m. p. 67°.

NN′-*Bishydroxymethyltrimethylenedinitroamine* (I; $n = 3$; R = $CH_2{\cdot}OH$).—Trimethylenedinitroamine (0·5 g.) was dissolved in warm 40% aqueous formaldehyde (1 c.c., 2 mols.), the solution cooled to 0°, and the product, which formed plates (0·58 g.), m. p. 88—92°, collected [Found: C, 26·1; H, 5·3; N, 25·3; CH_2O, 26·2; equiv., 111·5, 112·3. $C_5H_{12}O_6N_4$ requires C, 26·8; H, 5·4; N, 25·0; CH_2O (2 mols.), 26·8%; equiv., 112·0].

NN′-*Bismorpholinomethyltrimethylenedinitroamine* (I; $n = 3$; R = morpholinomethyl).—The preceding compound (0·5 g.) was dissolved in ethyl acetate (1 c.c.), and morpholine (0·5 c.c., 2 mols.) was added. After 1 hour at 0°, the solid *product* was collected and dried. Recrystallisation from ethyl acetate gave rosettes of prisms (0·68 g.), m. p. 130—132° [Found: C, 42·9; H, 7·0; CH_2O, 16·7, 17·2; equiv., 177·5. $C_{13}H_{26}O_6N_6$ requires C, 43·1; H, 7·2; CH_2O (2 mols.), 16·6%; equiv., 181].

NN′-*Biscarbomethoxytetramethylenediamine*.—Adipyl chloride (from 20 g. of adipic acid; cf. Fuson and Walker, *Org. Synth.*, 1933, **13**, 32) in an equal volume of chloroform was added slowly, with vigorous stirring, to aqueous ammonia (100 c.c., *d* 0·88). The product was collected and boiled in water containing a little charcoal until the chloroform had been removed; the filtered solution was cooled, and the adipamide (17 g.), m. p. 219°, collected. This was dissolved in a solution of bromine (13 c.c.) in 10N-sodium hydroxide (100 c.c.) and poured on ice (140 g.). After 1 hour the solution was heated under reflux for 3 hours, set aside overnight, and steam-distilled, the distillate being made acid with 2N-hydrochloric acid (120 c.c.) and evaporated to dryness. The residue of hydrochloride (9 g.) was collected and dissolved in 4N-sodium hydroxide (60 c.c.); the mixture was extracted with ether, the solvent removed, and the residual putrescine (3·0 g.) distilled, b. p. 140—150°/750 mm. (cf. Braun and Lemke, *Ber.*, 1922, **55**, *B*, 3549). The base was dissolved in water (10 c.c.), cooled in ice–salt, and stirred during the gradual addition of methyl chloroformate (6 c.c.). When about half of the latter had been added, 10N-sodium hydroxide (6·2 c.c.) was added at twice the rate, and, after the mixture had been kept for 2 hours at room temperature, *NN′*-biscarbomethoxytetramethylenediamine (4·0 g.), m. p. 128—130°, was collected, washed with water, and dried at 100°.

Tetramethylenedinitroamine (I; $n = 4$; R = H).—The preceding compound was converted into, successively, the *NN′*-dinitro-derivative and dinitroamine (cf. Dekkers, *Rec. Trav. chim.*, 1890, **9**, 96).

NN′-*Bishydroxymethyltetramethylenedinitroamine* (I; $n = 4$; R = $CH_2{\cdot}OH$).—Tetramethylenedinitroamine (I; $n = 4$; R = H) (0·6 g.) was dissolved in warm 40% aqueous formaldehyde (0·5 c.c.), and the solution kept overnight at 0°. The *product* was collected, washed with a little water, and dried (0·5 g.), m. p. 86—90° (Found: C, 30·4; H, 5·5; N, 23·6. $C_6H_{14}O_6N_4$ requires C, 30·3; H, 5·9; N, 23·5%).

NN′-*Bismorpholinomethyltetramethylenedinitroamine* (I; $n = 4$; R = morpholinomethyl).—The diol (I; $n = 4$; R = $CH_2{\cdot}OH$) (0·4 g.) was dissolved in a warm mixture of ethyl acetate (5 c.c.) and morpholine (0·3 c.c.). The solid *dinitroamine* (0·42 g.), which separated on cooling, crystallised from ethyl acetate in minute prisms, m. p. 156—159° (Found: C, 44·4; H, 7·3; N, 21·6. $C_{14}H_{28}O_6N_6$ requires C, 44·7; H, 7·7; N, 22·3%).

3 : 6-*Dinitro*-1-*oxa*-3 : 6-*diaza*cyclo*heptane* (IV).—*N*-Hydroxymethylethylenedinitroamine (5 g.) was heated at 140—145° for 1 hour in an open tube; formaldehyde and water were eliminated. The product was stirred with excess of sodium hydrogen carbonate solution, and the insoluble cyclic *compound* (0·85 g.), m. p. 150—152°, was collected, washed with water, and dried at 100°. It crystallised from hot water or acetone–benzene in a mass of fine needles, m. p. 154—155° (Found: C, 25·5; H, 4·3; N, 30·0; *M* (Rast), 187, 196. $C_4H_8O_5N_4$ requires C, 25·0; H, 4·2; N, 29·1%; *M*, 192). Acidification of the sodium hydrogen carbonate liquors and extraction with ethyl acetate, yielded ethylenedinitroamine, m. p. 170—172° (decomp.), undepressed by admixture with an authentic specimen.

NN′-*Bisnitroxymethylethylenedinitroamine* (I; $n = 2$; R = $CH_2{\cdot}O{\cdot}NO_2$).—The cyclic compound (IV) (0·5 g.) was added gradually with stirring to 98% nitric acid (5 c.c.) at −15°. After 10 minutes the mixture was added dropwise to anhydrous ether (50 c.c.) at −40°. The *diester* (0·64 g.) was collected, washed with dry ether, and dried; m. p. 112—117° (decomp.) (Found: C, 16·6; H, 2·7; $O{\cdot}NO_2$, 40·0; CH_2O, 19·8. $C_4H_8O_{10}N_6$ requires C, 16·0; H, 2·7; $O{\cdot}NO_2$, 41·3; CH_2O, 20·0%). At > −40°, reduced yields were obtained.

NN′-*Bismethoxymethylethylenedinitroamine* (I; $n = 2$; R = $CH_2{\cdot}OMe$).—The foregoing diester (0·1 g.) was heated under reflux with methyl alcohol (2 c.c.) for 0·5 hour, and the solution concentrated and allowed to cool. The *product* (0·07 g.), which separated on cooling and scratching, crystallised from methyl alcohol in nacreous plates, m. p. 79—80° (Found: C, 30·7; H, 5·9; N, 23·6. $C_6H_{14}O_6N_4$ requires C, 30·2; H, 5·9; N, 23·5%).

NN′-*Bisacetoxymethylethylenedinitroamine* (I; $n = 2$; R = $CH_2{\cdot}OAc$).—The dinitroxy-compound (I; $n = 2$; R = $\cdot CH_2{\cdot}O{\cdot}NO_2$) (0·16 g.) was heated under reflux for 5 minutes with sodium acetate (0·16 g.) and glacial acetic acid (1 c.c.), the solvent was removed in a vacuum, and the residue triturated with water. The resultant sticky gum, which gradually solidified, was collected, washed with water, and crystallised from ethyl alcohol. The product (0·1 g.), m. p. 67—70°, crystallised from glacial acetic acid in large colourless plates, m. p. 67—69°, undepressed by admixture with a specimen, m. p. 67—70°, prepared as described by Myers and Wright ("Nitrolysis of Hexamine, Part IV," *Canadian J. Res.*, in the press.)

The diacetoxy-compound (1·0 g.) was added to 98% nitric acid (10 c.c.) at 5° and, after 15 minutes, the nitration mixture was added dropwise to anhydrous ether (50 c.c.) at <10°. The micro-crystalline precipitate was collected, washed with dry ether, and dried in a vacuum; the product (0·42 g.) had m. p. 115—120° (decomp.), undepressed by admixture with the product, m. p. 112—117° (decomp.), obtained by nitration of compound (IV). The identity of the substance was further confirmed by boiling with methyl alcohol which yielded the dimethoxy-compound (I; $n = 2$; R = $CH_2{\cdot}OMe$), m. p. 79—80°.

Acetylation of Ethylenedinitroamine.—Finely powdered ethylenedinitroamine (1·5 g.) was stirred with acetic anhydride (25 c.c.) at 65° for 6 hours, the solution evaporated in a vacuum at <65°, and the residue treated with excess of sodium bicarbonate solution. The product (0·24 g.), m. p. 133—134° (decomp.), which was collected, washed with water, and dried at 100°, crystallised from acetone–ligroin (b. p. 40—60°) in nacreous plates, m. p. 135—136° (decomp.) (Found: C, 31·2; H, 4·2; N, 24·5. Calc. for $C_6H_{10}O_6N_4$: C, 30·8; H, 4·3; N, 23·9%). W. E. Bachmann (private communication) reports m. p. 132—133°.

The author's thanks are due to Messrs. F. Chapman and C. Lindley for their work on tetramethylene dinitroamine.

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354. *Studies on Nitroamines. Part IV. The Reaction of Nitroamines with Formaldehyde and Primary or Secondary Amines.*

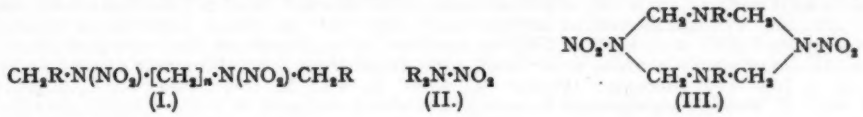
By F. CHAPMAN, P. G. OWSTON, and D. WOODCOCK.

A series of compounds (III; R = Me) and (V; R = *cyclo*hexyl or CH_2Ph) has been prepared from methylenedinitroamine, formaldehyde, and the appropriate base, the nitroamine acting in each case as a precursor of nitroamine (II; R = H). Under aqueous conditions, substances (IV; $n = 1$; R = Me, Et, or CH_2Ph) can be isolated, whilst there is evidence of the formation of (IV; $n = 1$; R = H) when ammonia is used. Compounds of type (IV) derived from ethylene- and trimethylene-dinitroamine are also described.

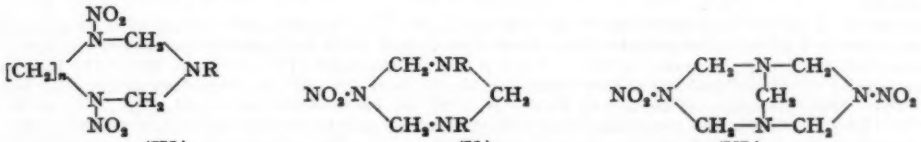
IN the preceding paper it was shown that compounds of type (I; R = morpholino; $n = 2$, 3, or 4) are formed from morpholine and the corresponding *N*-hydroxymethylnitroamine, but in the case of methylenedinitroamine the product obtained under aqueous or anhydrous conditions was (II; R = morpholinomethyl).

The reaction between methylenedinitroamine and other bases has now been examined. When a solution of methylenedinitroamine in ethyl acetate is treated with dry gaseous formaldehyde and then with an alcoholic solution of methylamine, the resultant product (III; R = Me) is identical with that obtained from nitroamine by Chute, Downing, McKay, Myers, and Wright ("Nitrolysis of Hexamine, Part I," *Canadian J. Res.*, in the press), and no methylenedinitroamine was regenerated on hydrolysis. On the other hand, the *product* from experiments in which 40% aqueous formaldehyde was used gave a theoretical yield of methylene-

dinitroamine (isolated as the insoluble barium salt); this hydrolysis and elementary analysis indicated that this latter product is (IV; $n = 1$; R = Me), and this was supported by synthesis from *NN'*-bishydroxymethylmethylenedinitroamine (I; $n = 1$; R = OH) and methylamine.



(I.) (II.) (III.)



(IV.) (V.) (VI.)

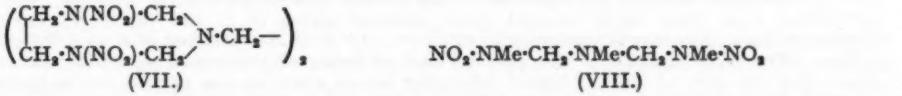
From methylenedinitroamine and *cyclo*hexylamine only one product, identical with that of Chute *et al.* (*loc. cit.*) (V; R = *cyclo*hexyl) could be isolated, whereas, when benzylamine was employed, a mixture of (IV; $n = 1$; R = benzyl) and (V; R = benzyl) was formed in proportions which depended on the conditions; the former component was obtained in 30% and 1—2% yield under aqueous and anhydrous conditions, respectively, but the eight-membered-ring compound (III; R = benzyl) was not obtained.

The isolation of compounds of types (II), (III), and (V) in which the grouping $\cdot CH_2 \cdot N(NO_2) \cdot CH_2 \cdot$ is derived from methylenedinitroamine, provides further evidence that the latter can act as a precursor of nitroamine.

It has been shown (Part VII) that the main product of the reaction, in aqueous solution, of methylenedinitroamine, formaldehyde, and ammonium ions, at pH 5—6 is 3 : 7-dinitro-1 : 3 : 5 : 7-tetra-aza*bicyclo*[3 : 3 : 1]nonane (VI). In the present investigation, treatment of a solution of methylenedinitroamine in anhydrous ether with a saturated solution of gaseous formaldehyde and ammonia in the same solvent gave a gummy product which probably contained (IV; $n = 1$; R = H) because its acetyl derivative (IV; $n = 1$; R = Ac) (Aristoff, Graham, Myers, and Wright, "Nitrolysis of Hexamine, Part VI," *Canadian J. Res.*, in the press; also Roberts, private communication) was isolated after boiling with acetyl chloride.

Of secondary amines investigated, other than morpholine, diethylamine and piperidine gave ill-defined products with methylenedinitroamine and formaldehyde.

The 1 : 5-dinitro-3-alkyl-1 : 3 : 5-triaza*cyclo*heptanes (IV; $n = 2$; R = *ethyl*, iso*propyl*, *butyl*, and cyclo*hexyl*) were prepared from ethylenedinitroamine and, unlike the corresponding derivatives of nitroamide and methylenedinitroamine, were stable in hot organic solvents. 3 : 3'-*Ethylenebis*-(1 : 5-*dinitro*-1 : 3 : 5-*triaza*cyclo*heptane*) (VII), prepared similarly from ethylenedinitroamine and ethylenediamine, is an extremely insoluble compound.



(VII.) (VIII.)

Under anhydrous conditions, trimethylenedinitroamine reacts with methylamine or *cyclo*hexylamine in the presence of formaldehyde to give *compounds* (IV; $n = 3$; R = Me and *cyclo*hexyl, respectively). The former product is more stable than the corresponding methylenedinitroamine derivative (IV; $n = 1$; R = Me), whilst the compound (IV; $n = 1$; R = *cyclo*hexyl) has not so far been obtained.

Methylnitroamine, formaldehyde, and methylamine under aqueous conditions yield the *compound* (VIII). A number of salts, prepared from methylenedinitroamine and organic bases for the purposes of comparison, are described in the experimental section.

Experimental.

1 : 5-*Dinitro*-3 : 7-*dimethyl*-1 : 3 : 5 : 7-*tetra*-*aza*cyclo*octane* (III; R = Me).—A solution of methylene dinitroamine (0·5 g.) in ethyl acetate (1 c.c.) was saturated with dry gaseous formaldehyde at 0°, and a 33% solution of methylamine in ethyl alcohol (0·9 c.c., 2 mols.) added with stirring. The solid (0·35 g.), m. p. 125—126° (decomp.), which separated, was collected, washed with ether, and dried in a vacuum (Found: C, 31·0; H, 6·0; N, 35·0; CH_2O, 49·1. Calc. for $C_6H_{14}O_4N_6$: C, 30·8; H, 6·0; N, 35·8; CH_2O, 51·2%). The solid was sparingly soluble in cold water, evolving formaldehyde on warming, and soluble in 2N-hydrochloric acid. Methylenedinitroamine could not be isolated after hydrolysis

with cold barium hydroxide solution, and admixture with the compound, m. p. 124° (decomp.), as prepared by Wright (*loc. cit.*) from nitroamine, gave no depression in m. p.

1-*Nitro*-3 : 5-*dibenzylhexahydro*-1 : 3 : 5-*triazine* (V; R = CH_2Ph).—This was prepared as above, using benzylamine (0·8 c.c.) instead of methylamine. After the mixture had been kept overnight at 0°, the white solid (0·63 g.), m. p. 109—115° (decomp.), was collected, dried in a vacuum, and crystallised from cold acetone. A small amount of material, m. p. 130—131° (decomp.), undepressed by admixture with the compound (IV; $n = 1$; R = CH_2Ph) described below, separated first, and addition of water to the mother-liquors gave a white solid which crystallised from a small amount of acetone in small needles, m. p. 108—110° (decomp.) (Found: C, 65·6; H, 6·3; N, 18·1. Calc. for $C_{17}H_{20}O_2N_4$: C, 65·4; H, 6·5; N, 18·0%), undepressed by admixture with compound (V; R = CH_2Ph) obtained from nitroamine.

1-*Nitro*-3 : 5-*di*cyclo*hexylhexahydro*-1 : 3 : 5-*triazine* (V; R = *cyclo*hexyl).—A solution of methylenedinitroamine (0·7 g.) in ethyl acetate (1·5 c.c.) was saturated with dry gaseous formaldehyde at 0°, and *cyclo*hexylamine (1·0 g., 2 mols.) added. After 0·5 hour the solid (1·0 g.), m. p. 99—101° (decomp.), was collected; it crystallised from ether–light petroleum (b. p. 40—60°) in colourless rectangular prisms, m. p. 101—102° (decomp.) (Found: C, 61·0; H, 9·2; N, 18·6. Calc. for $C_{15}H_{26}O_2N_4$: C, 60·9; H, 9·5; N, 18·9%). The same compound was obtained using excess of 40% aqueous formaldehyde, and neither substance depressed the m. p. of a product, m. p. 99° (decomp.), prepared by Wright's method (*loc. cit.*).

1 : 5-*Dinitro*-3-*benzylhexahydro*-1 : 3 : 5-*triazine* (IV; $n = 1$; R = CH_2Ph).—Methylenedinitroamine (0·5 g.) was dissolved in 40% aqueous formaldehyde (1·5 c.c., 5 mols.), the solution cooled to 0°, and benzylamine (0·8 c.c., 2 mols.) added dropwise with shaking. After 18 hours at 0°, the white solid *triazine* (1·28 g.), m. p. 104—114° (decomp.), was collected and dried in a vacuum. Crystallisation from cold acetone gave rectangular prisms (0·2 g.), m. p. 127—130° (decomp.) (Found: C, 45·3, 45·2; H, 4·8, 4·9; N, 26·6, 26·4. $C_{10}H_{13}O_4N_5$ requires C, 45·0; H, 4·9; N, 26·2%). Addition of water to the mother-liquors gave a white solid which crystallised from cold acetone in needles, m. p. 107—110° (decomp.), undepressed by admixture with (V; R = CH_2Ph) prepared as described above.

1 : 5-*Dinitro*-3-*methylhexahydro*-1 : 3 : 5-*triazine* (IV; $n = 1$; R = Me).—(*a*) A solution of methylenedinitroamine (1·0 g.) in 40% aqueous formaldehyde (1 c.c., 3 mols.) was cooled to 0°, and a 33% ethyl-alcoholic solution of methylamine (0·9 c.c., 1 mol.) was quickly added with shaking. After 1 hour at 0°, the crystalline *triazine* (0·6 g.), m. p. 100—104° (decomp.), was collected, washed with cold water, and dried in a vacuum [Found: C, 26·0; H, 4·8; N, 35·4; potential methylenedinitroamine (see Part VII), 70·5, 71·2. $C_4H_9O_4N_5$ requires C, 25·2; H, 4·7; N, 36·7; potential methylenedinitroamine, 71·2%). (*b*) A solution of *NN'*-bishydroxymethylmethylenedinitroamine (0·15 g.) in ethyl acetate (0·5 c.c.) was cooled to 0°, and a 33% ethyl-alcoholic solution of methylamine (0·1 c.c., 1 mol.) added. After 2 hours at 0°, large colourless rhombs (0·08 g.), m. p. 98—99° (decomp.), undepressed by admixture with product (*a*) above, were collected, washed with ether, and dried in a vacuum.

1 : 5-*Dinitro*-3-*ethylhexahydro*-1 : 3 : 5-*triazine* (IV; $n = 1$; R = Et).—A solution of methylenedinitroamine (1·0 g.) in 40% aqueous formaldehyde (1·0 c.c., 3 mols.) was cooled to <0°, and ethylamine (0·25 c.c., 1 mol.) added dropwise with shaking. The white crystalline *product* (1·0 g.), m. p. 88—89° (decomp.), was collected and dried in a vacuum (Found: C, 29·4; H, 5·3; N, 34·1; potential methylenedinitroamine, 65·5. $C_5H_9O_4N_5$ requires C, 29·3; H, 5·4; N, 34·1; methylenedinitroamine, 66·3%). It was soluble in cold water, but decomposed rapidly when warmed. Hydrolysis with barium hydroxide yielded methylenedinitroamine.

1 : 5-*Dinitro*-3-*acetylhexahydro*-1 : 3 : 5-*triazine* (IV; $n = 1$; R = Ac).—Anhydrous ammonia in ether (2 c.c.; 1 mol.-equiv. of NH_3), prepared by saturation at −10°, was added cautiously with stirring to a solution of methylenedinitroamine (0·5 g.) in ethyl acetate (1 c.c.) which had been previously treated at 0° with excess of dry gaseous formaldehyde. The sticky white solid (*A*) obtained by decanting the solvent was heated under reflux for 0·5 hour with an excess of acetyl chloride, the solution cooled, and the insoluble material (*B*) collected. The filtrate was evaporated to dryness; the residual oil, crystallised twice from methyl alcohol, gave prismatic plates, m. p. 156° (decomp.), not depressed by admixture with an authentic specimen of (IV; $n = 1$; R = Ac) (Aristoff *et al.* and Roberts, *locc. cit.*). *X*-Ray diffraction diagrams of this product and of Roberts's compound were identical. Product (*B*) which was the only product obtained when the above reaction was carried out entirely in ethereal solution, was insoluble in water and failed to melt below 360°, though partly subliming. Done in methyl alcohol, the reaction yielded an intractable amorphous material which gradually sublimed at >220°. An attempt to prepare the nitrate of the base (IV; R = H) by treating the substance (*A*) with 55% nitric acid failed.

Methylbis-(N-*nitro*-N-*methylaminomethylamine*) (VIII).—A 33% aqueous solution of methylamine (1·4 c.c., 1 mol.) was added dropwise, with shaking, to a solution of methylnitroamine (2·0 g.) in 40% aqueous formaldehyde (2·0 c.c., 2 mols.) at <0°. The oil which separated from the clear solution gradually solidified (1·42 g.) and was collected, washed with water, and dried in a vacuum; from the mother-liquors the *product* was obtained in large colourless hexagonal prisms, m. p. 62—63° (Found: C, 29·2; H, 6·3; N, 32·9. $C_5H_{12}O_4N_6$ requires C, 29·3; H, 6·3; N, 33·8%).

1 : 5-*Dinitro*-3-*methyl*-1 : 3 : 5-*triaza*cyclo*octane* (IV; $n = 3$; R = Me).—A solution of trimethylenedinitroamine (0·5 g.) in ethyl acetate (0·5 c.c.) was saturated at −10° with dry gaseous formaldehyde, and 33% ethyl alcoholic methylamine (0·75 c.c., 2 mols.) was added slowly with shaking. The 1 : 3 : 5-*triaza*cyclo*octane* (0·62 g.), m. p. 141—143°, was collected and dried in a vacuum (Found: C, 33·0; H, 5·9; N, 32·1; CH_2O, 30·0, 30·7. $C_6H_{12}O_4N_6$ requires C, 32·8; H, 5·9; N, 31·9; CH_2O, 27·4%).

1 : 5-*Dinitro*-3-cyclo*hexyl*-1 : 3 : 5-*triaza*cyclo*octane* (IV; $n = 3$; R = cyclo*hexyl*).—The preceding experiment was repeated using *cyclo*hexylamine (0·3 g., 1 mol.), and the *product* (0·4 g.) was collected, washed with ether, and dried in a vacuum. Recrystallisation from methyl alcohol gave rosettes of prisms, m. p. 144—145° (decomp.) (Found: C, 46·0; H, 7·1; N, 26·4. $C_{11}H_{21}O_4N_5$ requires C, 46·0; H, 7·3; N, 24·4%).

1 : 5-*Dinitro*-3-*alkyl*-1 : 3 : 5-*triaza*cyclo*heptanes* (IV; $n = 2$; R = alkyl).—These *compounds* were

prepared by Wright's method (*loc. cit.*) described for the 3-methyl compound; data below include the yield from ethylenedinitroamine (1·0 g.). 3-Ethyl: rectangular plates (1·0 g.), m. p. 140—142°(from ethyl acetate) (Found: C, 32·9; H, 5·1; N, 33·1. $C_5H_{10}O_4N_5$ requires C, 33·0; H, 5·9; N, 32·0%). 3-*iso*Propyl: minute prisms (0·7 g.), m. p. 107—108° (from ethyl alcohol or ethyl acetate) (Found: C, 36·2; H, 6·1; N, 28·7. $C_7H_{13}O_4N_5$ requires C, 36·1; H, 6·4; N, 27·4%). 3-*n*-Butyl: long prisms (1·1 g.), m. p. 106—107° (from carbon tetrachloride–acetone) (Found: C, 38·3; H, 6·8; N, 27·8. $C_8H_{17}O_4N_5$ requires C, 38·3; H, 6·9; N, 28·4%). *cyclo*Hexyl: clusters of prisms (0·5 g.), m. p. 127° (from ethyl acetate) (Found: C, 44·2; H, 7·1; N, 26·2. $C_{10}H_{19}O_4N_5$ requires C, 44·0; H, 7·0; N, 25·6%).

3 : 3′-*Ethylenebis*-(1 : 5-*dinitro*-1 : 3 : 5-*triaza*cyclo*heptane*) (VII).—This *compound* (1·3 g.), prepared similarly from ethylenedinitroamine (1·0 g.) and ethylenediamine hydrate (0·3 g.), was microcrystalline, had m. p. 205—207° (decomp.), was insoluble in the usual organic solvents, and was purified by washing with sodium hydrogen carbonate solution, water, and finally acetone (Found: C, 29·9; H, 4·9; N, 33·2. $C_{10}H_{20}O_8N_{10}$ requires C, 29·4; H, 4·9; N, 34·3%).

Salts of Methylenedinitroamine.—(*a*) To a cold solution of methylenedinitroamine (0·5 g.) in ethyl acetate (1 c.c.) 33% methylamine in ethyl alcohol (0·9 c.c., 2 mols.) was added. After 0·5 hour at 0°, the very hygroscopic *dimethylamine* salt was collected, recrystallised from methyl alcohol containing a little methylamine, washed with anhydrous ether, and dried in a desiccator, after which it had m. p. 120° (decomp.) (Found: C, 19·0; H, 7·0; N, 39·1. $C_3H_{11}O_4N_6$ requires C, 18·2; H, 7·1; N, 42·4%).

(*b*) The *di*cyclo*hexylamine* salt, similarly prepared from *cyclo*hexylamine (0·85 c.c., 2 mols.), had m. p. 99—100° (decomp.) after recrystallisation from methyl alcohol (Found: C, 46·6; H, 8·9; N, 24·1. $C_{13}H_{30}O_4N_6$ requires C, 46·8; H, 9·0; N, 25·2%).

(*c*) The *dibenzylamine* salt, similarly prepared from benzylamine, crystallised from methyl alcohol containing a trace of benzylamine in aggregates of stout prisms, m. p. 112° (decomp.) (Found: C, 51·8; H, 6·2; N, 24·0. $C_{15}H_{22}O_4N_6$ requires C, 51·4; H, 6·4; N, 24·0%).

The authors' thanks are due to Professor F. J. Llewellyn for the *X*-ray photographs.

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355. *Studies on Nitroamines. Part V. Some Properties of Hydroxymethyl- and Aminomethyl-nitroamines.*

By ALEX. H. LAMBERTON, C. LINDLEY, P. G. OWSTON, and J. C. SPEAKMAN.

In Parts III and IV of this series, the production of a number of compounds of types (I) and (II) has been reported. Their mode of formation and chemical individuality is now considered, the following conclusions being drawn: (*a*) in aqueous solution, hydroxymethylnitroamines (I) react predominantly as mixtures of formaldehyde and the nitroamine, but they have greater stability in mineral acid; (*b*) compounds of type (I) are not precursors of type (II); reaction probably proceeds by decomposition of type (I), followed by formation of the hydroxymethyl amine ($NR'_2{\cdot}CH_2{\cdot}OH$), and union thereof with the nitroamine; (*c*) aminomethylnitroamines (II) decompose, on (or after) dissolution in water, to formaldehyde and salts of the nitroamine anion with the amino-cation. In alkaline solution substances of type (II) yield the nitroamine anion and an equilibrium mixture of amine, formaldehyde, and hydroxymethylamine, whilst in mineral acid they are stabilised, possibly by formation of the cation $NO_2{\cdot}NR{\cdot}CH_2{\cdot}\overset{\oplus}{N}HR'_2$.

AT an early stage in the progress of the work detailed in Parts III and IV of this series it became apparent that parallel consideration should be given to the mechanism of formation and to the stability of compounds of types (I) and (II). The results of this work are best discussed and interpreted in terms of the reversible reactions (*A*), (*B*), and (*C*).

$$\underset{\text{(I.)}}{NO_2{\cdot}NR{\cdot}CH_2{\cdot}OH} \qquad \underset{\text{(II.)}}{NO_2{\cdot}NR{\cdot}CH_2{\cdot}NR'_2}$$

$$(A)\quad NHR{\cdot}NO_2 + CH_2O \rightleftharpoons NO_2{\cdot}NR{\cdot}CH_2{\cdot}OH$$

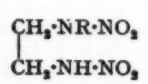
$$(B)\quad NHR'_2 + CH_2O \rightleftharpoons NR'_2{\cdot}CH_2{\cdot}OH$$

$$(C)\quad NHR{\cdot}NO_2 + CH_2O + NHR'_2 \rightleftharpoons NO_2{\cdot}NR{\cdot}CH_2{\cdot}NR'_2$$

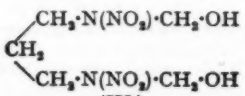
$$(D)\quad \begin{matrix} OH \\ CH_2 \\ X \end{matrix} \overset{-H^\oplus}{\underset{+H^\oplus}{\rightleftharpoons}} \begin{matrix} \overset{\ominus}{O} \\ CH_2 \\ X \end{matrix} \rightleftharpoons X^\ominus + CH_2O$$

Reaction A.—The formal analogy between cyanohydrins (see Lapworth *et al.*, *J.*, 1930, 448; 1931, 1387) and hydroxymethylnitroamines is illustrated in equation *D* (X = ·NR·NO_2 or CN). The nitroamine ion is, however, relatively more stable than the cyanide ion (the K_a

values for hydrogen cyanide and methylnitroamine are 5×10^{-10} and 5×10^{-7}, respectively), and it has been found that, unlike hydrogen cyanide which reacts with cotarnine to give a ψ-cyanide, methylnitroamine and nitrourethane yield cotarninium salts in the form of water-soluble gums (curves II*a*, II*b*, Fig. 2). It would therefore be expected that the equilibrium in equation *A* would lie more to the left than in the case of the formation of cyanohydrins. In fact, aqueous solutions of the compounds (III; R = CH_2·OH) and (IV) can be accurately



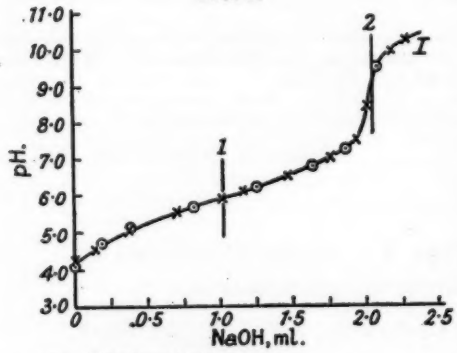
(III.)



(IV.)

and directly titrated with sodium hydroxide, using phenolphthalein as indicator (Part III). Furthermore, it has now been shown that the formaldehyde of both (III; R = CH_2·OH) and (IV) can be determined accurately in cold water by means of iodine and alkali, whilst a dilute aqueous solution of (III; R = CH_2·OH) is indistinguishable, on potentiometric titration, from a mixture of formaldehyde and ethylenedinitroamine (curve 1, Fig. 1).

FIG. 1.

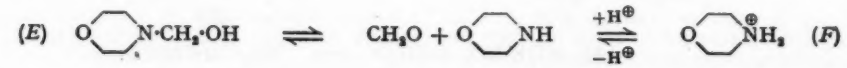


I { ⊙ *Ethylenedinitroamine.*
× *Compound (III*; R = CH_2·OH).
— *Also in presence of* 1 *equiv. excess of formaldehyde.*

In contrast to the stability of cyanohydrins in acid solution, the decomposition of hydroxymethylnitroamines (equation *D*; X = NR·NO_2) appears to be only partly suppressed, presumably on account of the greater stability of the nitroamine ion. When aqueous solutions of (III; R = CH_2·OH) or (IV) were acidified and treated with Brady's reagent (2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid), quantitative precipitation of the formaldehyde derivative occurred, but, if the solid compounds were dissolved in 2N-hydrochloric acid instead of water, the yield of hydrazone precipitated under the same conditions fell to 50% and 60%, respectively.

It would be too sweeping, however, to conclude that in neutral or alkaline solutions the interaction of formaldehyde and nitroamines may always be disregarded (Myers and Wright, "Nitrolysis of Hexamine, Part IV," *Canadian J. Res.*, in the press). Further, Chute, Downing, McKay, Myers, and Wright (*ibid.*, Part I) have drawn attention to the stabilisation of nitroamine by formaldehyde, and we have found that, in the presence of formaldehyde, nitroamine can be titrated with sodium hydroxide to a moderately stable end-point, whilst, conversely, the formaldehyde is shielded to some extent from precipitation as the 2 : 4-dinitrophenylhydrazone.

Reaction B.—The reversibility of this reaction is generally accepted. Potentiometric measurements now reported show that the decrease in basicity observed after the addition of formaldehyde to morpholine solutions may be accounted for quantitatively in terms of the following scheme :



(*E*) (*F*)

When solutions of morpholine hydrochloride and formaldehyde are mixed and treated with Brady's reagent, the hydrazone is at once precipitated; if, however, formaldehyde is allowed to react first with morpholine base, hydroxymethylmorpholine is produced and, after rapid addition of a large excess of acid, the precipitation of the hydrazone is delayed and scanty. This retention of formaldehyde is not observed after slow addition of N-acid or after the rapid addition of only one or two equivalents of 0·06N-acid.

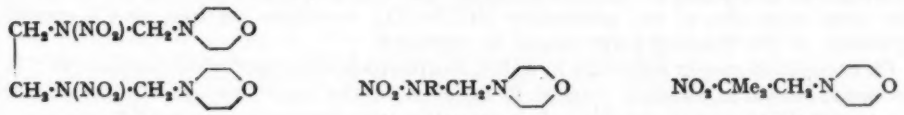
These results may be satisfactorily correlated. Morpholine hydrochloride does not react with formaldehyde, the carbonyl group having presumably no affinity for the morpholinium ion. Morpholine does react and, on rapid acidification, salts of both morpholine and hydroxymethylmorpholine are formed; the hydroxymethylmorpholine salt may be relatively stable in

acid solution (cf. Robinson and Robinson, *J.*, 1932, 532, who comment on the stability of $BuO{\cdot}CH_2{\cdot}\overset{\oplus}{N}R_2$ in contrast to $BuO{\cdot}CH_2{\cdot}NR_2$). On slow acidification, the stronger base (morpholine) engages the limited amount of acid, equilibrium *E* is disturbed, and formaldehyde is freed.

Reaction C.—It was pointed out by Lapworth *et al.* (*loc. cit.*) that the electron-restraining properties of the nitrile group should inhibit the removal of the hydroxyl ion from cyanohydrins, and thus (*a*) prevent the preparation of derivatives of malononitrile by the action of cyanide ions on cyanohydrins and (*b*) entail an indirect formation of α-amino-nitriles from cyanohydrins and secondary bases. Similarly, hydroxymethylnitroamines (type I) may resist elimination of the hydroxyl ion. The failure of many workers to prepare cyclonite from nitroamine and formaldehyde (private communications) is thus comprehensible, and the reactions which occur when a preformed hydroxymethylnitroamine (I) is treated with a secondary base very possibly involve decomposition to nitroamine and formaldehyde (equation *A*), formation of the amino-methyl alcohol (equation *B*), and condensation of the nitroamine and aminomethyl alcohol by process (*G*). Myers and Wright (" Nitrolysis of Hexamine, Part IV," *loc. cit.*), however, favour the direct condensation of hydroxymethylnitroamine and base.

$$(G)\quad NHR{\cdot}NO_2 + HO{\cdot}CH_2{\cdot}NR'_2 \longrightarrow NO_2{\cdot}NR{\cdot}CH_2{\cdot}NR'_2 + H_2O$$

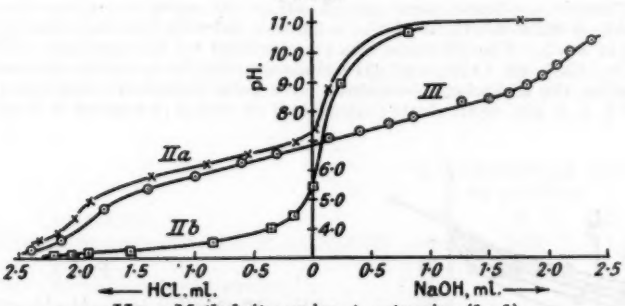
It is difficult to decide this point experimentally, but compound (V) appears to be precipitated more rapidly when, in place of separate addition, a preformed mixture of morpholine and formaldehyde is added to an aqueous solution of the morpholine salt of ethylenedinitroamine.



(V.) (VI.) (VII.)

Compounds (III; R = morpholinomethyl) and (V) (Part III), morpholinomethylmethyl-nitroamine (VI; R = Me), and the aminomethylnitroamines (VI; R = CO_2Et or $CO{\cdot}NH_2$) are

FIG. 2.



IIa. Methylnitroamine + cotarnine (1 : 1).
IIb. Nitrourethane + cotarnine (1 : 1).
III. Compound V.

water-soluble in varying degree, but very sparingly soluble in ether or benzene. They can be titrated directly with sodium hydroxide, using thymolphthalein as indicator, and, with the single exception of compound (VI; R = $CO{\cdot}NH_2$), the methylene groups can be accurately determined as formaldehyde by iodine in cold alkaline solution. By contrast, 4-2′-nitro*iso*-butylmorpholine (VII), since prepared independently by Johnson (*J. Amer. Chem. Soc.*, 1946, **68**, 13), was found to be a typically covalent compound, insoluble in water, easily soluble in ether and benzene, and not titratable with alkali.

Potentiometric titrations have been carried out with sodium hydroxide and hydrochloric acid on dilute solutions of compound (V). The pH–neutralisation curve obtained (curve III, Fig. 2) resembles that of a salt of a weak acid and a weak base, and indicates that decomposition follows (or accompanies) dissolution in water. The positive ion (or ions), studied during titration with sodium hydroxide, behaves as an effectively stronger acid than the morpholinium ion (*i.e.*, morpholine appears to have been converted into an effectively weaker

base), its apparent strength varies during the course of the titration, and the results can be interpreted in terms of equations (*E*) and (*F*). The aminomethylnitroamines are therefore assumed to be effectively decomposed in aqueous solution by reversal of equation (*G*), followed by equilibration of the aminomethyl alcohol according to equations (*E*) and (*F*); and compounds (III; R = morpholinomethyl) and (V) then yield more than 80% of their formaldehyde on treatment with Brady's reagent. If, however, these solids are dissolved in 2N-hydrochloric acid, no hydrazone is precipitated in 5 hours, and only 10—20% of the theoretical amount separates in 24 hours. This retention of formaldehyde in strongly acid solution might be due to the formation of the stable cation of hydroxymethylmorpholine (see discussion of reaction *B*), or to the formation, without rupture, of the stable cation (VIII).

$NO_2 \cdot NR \cdot CH_2 \cdot \overset{\oplus}{H}N$ (morpholine ring) O

(VIII.)

A decision between these alternatives was made by cryoscopic determinations of molecular weight in N-hydrochloric acid. The freezing point of hydrochloric acid is lower than that of water, on account of the ions which the solution contains. If some soluble material is now added, the total number of dissolved particles is normally increased; a further depression of the freezing point is thus produced, from which the molecular weight of the added compound could be calculated. However, if an aminomethylnitroamine is added, it may react as a simple amine and combine with the protons in the solution to yield ammonium cations (VIII); the total number of dissolved particles will not then be augmented. If no other reaction occurs and salt formation is complete, no further depression of the freezing point is to be expected and the molecular weight of the added aminomethylnitroamine would apparently be infinite. Formation of the cation of hydroxymethylmorpholine would also take up protons, but at the same time molecules of the nitroamine ($NHR \cdot NO_2$) would be set free, and a corresponding depression of the freezing point might be expected.

On account of greater solubility in water, morpholinomethylmethylnitroamine (VI; R = Me) was used; towards Brady's reagent it behaved in the same way as the compounds (III; R = morpholinomethyl) and (V). In water, the apparent molecular weight was one third of the formula weight, the decomposition of these substances in accordance with equation (*C*) being thus confirmed; but the high apparent molecular weight in acid suggested that the compound existed mainly as the cation (VIII).

Experimental.

Potentiometric Titrations.—These were carried out at 20° using the glass electrode; 0·06M-titrant was run in to 0·001—0·003M-solutions of the substance investigated, and formaldehyde was used as 0·05M-solution of pH 6—7. The pH-scale was standardised by the method of Hitchcock and Taylor (*J. Amer. Chem. Soc.*, 1937, **59**, 1812), and pH values are reliable to within 0·02 unit. Details and the methods of calculating the dissociation constant have been described elsewhere (*J.*, 1940, 855).

The addition of ½, 1, 2, etc. equivalents (calculated) of titrant is marked in Figs. 1, 2, and 3 by short vertical lines.

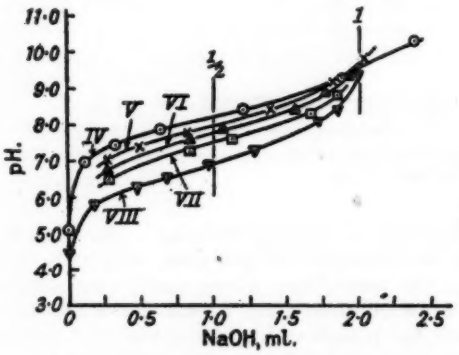


Fig. 3.

IV. *Morpholine hydrochloride alone.*
V. ,, ,, +½ *mole formaldehyde.*
VI. ,, ,, +1 ,, ,,
VII. ,, ,, +2 *moles* ,,
VIII. ,, ,, +7 ,, ,,

The correspondence of curve VI, Fig. 3, at pH > 7·5, with curve III, Fig. 2, shows that any interaction between morpholine and formaldehyde is probably the same in both cases. The curves (Fig. 3) show that formaldehyde renders morpholine an effectively weaker base, but, in agreement with the greater slopes of curves V, VI, VII, and VIII, as compared with IV, the apparent dissociation constant varies during the titration. The effect of formaldehyde on morpholine is progressive and does not cease with the addition of 1 mole of formaldehyde, and it is concluded that the interaction of formaldehyde with morpholine does not go to completion. Further investigation was made by titrating a solution containing morpholine and its hydrochloride with formaldehyde. The results confirmed those shown in Fig. 3, but it was found that 4-hydroxymethylmorpholine was not produced

instantaneously; pH values became constant only 2—5 minutes after each addition of sodium hydroxide or formaldehyde. More exact data were therefore obtained by making up solutions (containing known amounts of morpholine, its hydrochloride, and formaldehyde) separately, and measuring the pH values at leisure. Results are shown in Table I, in which a, b, and f are, respectively, the molar concentrations of morpholine hydrochloride, sodium hydroxide, and formaldehyde; $[H^+]$ and $[OH^-]$ were calculated from the pH by the relations, $pH = -\log_{10}[H^+]f_1$ and $[H^+] \times [OH^-] = 0{\cdot}68 \times 10^{-14}$, f_1 being the activity coefficient for a univalent ion calculated by the Debye–Hückel limiting law.

The results can be accounted for in terms of the equations E and F. Neglecting activity effects (these experiments were carried out at constant ionic strength), these equilibria lead to the relation

$$K_i = K_a . A\{F/[H^+] + A(1 + [H^+]/K_a)(K_a/[H^+]^2)\}(B - K_a A/[H^+]),$$

where K_i = the instability constant (equation E) of hydroxymethylmorpholine, K_a = the acidity constant (equation F) of the morpholinium ion (which may be estimated from the data represented by Curve IV, Fig. 3, as $2{\cdot}8 \times 10^{-9}$), $A = a - b + [OH^-]$, $B = b - [OH^-]$, and $F = f - a$.

TABLE I.

	a = 0·00342.							
	b = 0·00141.				b = 0·00204.			
f	0·00000	0·00169	0·00346	0·00678	0·00000	0·00345	0·00684	0·01360
$10^8 \times [H^+]$	0·414	0·865	1·50	2·80	0·194	0·656	1·26	2·34
$10^6 \times [OH^-]$	0·0175	0·85	0·49	0·26	0·038	0·011	0·59	0·29
$10^3 \times K_i$	—	0·80	0·89	0·92	—	0·82	0·90	0·97

Calculated values of K_i are constant within experimental error, thus indicating that the above data are consistent with equations E and F.

The reactions occurring during the titrations shown in Fig. 3 were proved reversible. Morpholine hydrochloride and formaldehyde (1 : 1) were titrated with sodium hydroxide to pH 10·5 as in Curve VI; no deviation from the curve was observed on back-titration with acid. Reversibility on rapid (but mild) acidification was also found by adding, to a similar solution of morpholine hydrochloride and formaldehyde, sodium hydroxide to pH 10, followed at once by hydrochloric acid to pH 2; subsequently, the titration curve with sodium hydroxide, when the pH value exceeded 5·5, did not differ appreciably from Curve VI.

Estimation of Potential Formaldehyde.—The formaldehyde derived from 0·05—0·1 g. of material was determined by six methods; the results of three of these are given in Table II.

Method A (direct Romijn titration). The material was dissolved in water, and the formaldehyde determined by treatment with an excess of iodine and of sodium hydroxide.

Method B. The material was dissolved in water (20 ml.) and, after eighteen hours, 10N-hydrochloric acid (5 ml.) and 2 : 4-dinitrophenylhydrazine (50 ml. of a saturated solution in 2N-hydrochloric acid) were added. After five hours at 0° the hydrazone was collected and weighed.

Method C. The material was dissolved in 2N-hydrochloric acid (25 ml.); after 18 hours, Brady's reagent was added and the hydrazone collected as described in method *B*.

TABLE II.

Compounds.	% Formaldehyde found by methods *A*.	*B*.	*C*.	Calc. % of formaldehyde.
N-Hydroxymethylethylenedinitroamine (III; R = CH_2·OH)	16·2 16·5	16·1	7·8	16·7
NN'-Dihydroxymethyltrimethylenedinitroamine (IV)	26·2 26·6	26·2	17·5	26·8
N-Morpholinomethylethylenedinitroamine (III; R = morpholinomethyl)	12·3 12·4	11·6	nil	12·0
NN'-Dimorpholinomethylethylenedinitroamine (V)	17·2 17·3	14·6	nil	17·2

Notes on alternative methods tested. Preliminary boiling under reflux (20 minutes) with water, followed by a Romijn determination, gave satisfactory results similar to those of method *A*. Distillation of formaldehyde from dilute sulphuric acid was unsatisfactory; blank determinations using ethylenedinitroamine showed that acetaldehyde (identified by formation of iodoform and of the 2 : 4-dinitrophenylhydrazone) was produced. Attempts to determine the formaldehyde by the use of sodium hydrogen sulphite were unsuccessful.

Interaction of Nitroamine and Formaldehyde.—Nitroamine (28—37 mg. of theoretical equiv. 62; "Inorg. Syntheses," Vol. I, 468) was dissolved in water (10 ml.) and at once titrated (during 1, 2, or 3½ minutes) with sodium hydroxide, to thymolphthalein. Equivalents of 65, 68, and 71, respectively were obtained. Dissolution in 0·2M-formaldehyde (10 ml.) and titration during 2 minutes gave equivalents of 64, 65, and 65. The stabilising effect of the formaldehyde was marked at the end-point of the titrations, when the alkaline solutions were set aside, and the extent of the nitroamine decomposition measured by back-titration with standard acid. For example, in water alone 38% of the titrated acidity had disappeared in about 35 seconds, and 97% in 6 minutes, whilst keeping in formaldehyde for the same periods led to losses of only 1% and 56%, respectively.

Nitroamine was also observed to shield formaldehyde from precipitation as the 2 : 4-dinitrophenylhydrazone. Aliquots (10 ml.) of a standard solution of formaldehyde [formaldehyde content; 9·3, 9·2 mg. (iodometrically), and 9·5 mg. (Brady)] were added to nitroamine (10—12 mg., freshly dissolved in 10 ml. of water), and the resultant mixtures titrated with sodium hydroxide. After the end-point had been attained, the solutions were re-acidified (with 5 ml. of concentrated hydrochloric acid) either immediately or after standing for a time (25 minutes) known to suffice for the destruction of nitroamine in the presence of formaldehyde. By precipitation with Brady's reagent, the formaldehyde found was 9·4 or 9·5 mg. after destruction of the nitroamine, but only 6·8 or 7·0 mg. after retention of the nitroamine by immediate acidification.

Action of Morpholine on Formaldehyde.—An equilibrium mixture of morpholine and formaldehyde was prepared from standard solutions of 0·06M-morpholine hydrochloride (5 ml.), 0·05M-formaldehyde (5 ml.), and 0·095M-sodium hydroxide (3 ml.). On rapid acidification with hydrochloric acid (30 or more equivalents of at least 1M-acid) and treatment with Brady's reagent, precipitation of the hydrazone commenced only after 10 minutes, and was slight even after 24 hours. If, as an intermediate treatment, hydrochloric acid was added slowly (2 equivalents in 35 minutes) or the mixture was set aside with one or two equivalents of acid, precipitation commenced after 15—35 seconds under comparable final conditions of acidity.

N-*Morpholinomethylmethylnitroamine* (VI; R = Me).—38% Formaldehyde (0·5 ml.) and morpholine (0·5 ml.) were added to methylnitroamine (0·4 g.), and the mixture cooled to 0°. The *morpholinomethyl* compound (0·55 g.), which rapidly separated as rhombic prisms, m. p. 84—86°, was collected and washed with ice-water (2 ml.), alcohol, and ether [Found: C, 41·1; H, 7·6; CH_2O (direct Romijn titration), 17·5, 16·8; equiv., 178. $C_6H_{12}O_3N_2$ requires C, 41·4; H, 7·5; CH_2O, 17·1%; equiv., 175].

N-*Morpholinomethylnitrourethane* (VI; R = CO_2Et).—Morpholine (1 ml.) was added with water-cooling to nitrourethane (1·3 g.) in water (2 ml.). The salt solution was filtered, and 38% formaldehyde (1 ml.) added; after 30 minutes, scratching induced crystallisation of the *monohydrate*. The solid was collected, washed with ice-water, acetone, and ether, and air-dried at room temperature; prisms (1·5 g.), m. p. 85—86°, were obtained [Found: C, 38·5; H, 6·8; CH_2O (by direct Romijn titration), 11·6; equiv., 250, 249. $C_8H_{15}O_5N_3,H_2O$ requires C, 38·2; H, 6·8; CH_2O, 11·9%; equiv., 251]. The product, which was easily soluble in water or alcohol, moderately in hot chloroform or ethyl acetate, and insoluble in ether or benzene, crystallised from ethyl acetate, but the hot solution had an odour of formaldehyde, and the crystallised material was no purer than that precipitated directly from water. Formaldehyde was also eliminated on heating the substance or drying it in a vacuum-desiccator. The *morpholine* salt of nitrourethane, prepared for comparison by admixture, with cooling, of morpholine (0·1 ml.) and nitrourethane (0·13 g.), crystallised from ethyl acetate (5 ml.) in needles (0·2 g.), m. p. 75° (decomp.), which were easily soluble in water, soluble in alcohol or chloroform, and insoluble in ether or cold ethyl acetate (Found: C, 38·0; H, 7·0. $C_7H_{15}O_5N_3$ requires C, 38·0; H, 6·8%).

Morpholinomethylnitrourea (VI; R = $CO{\cdot}NH_2$).—The method described for *N*-morpholinomethylnitrourethane, but with the substitution of nitrourea (1 g. in 4 ml. of water) for the aqueous solution of nitrourethane, yielded the *morpholinomethyl* compound as prisms (1·5 g.), m. p. 120° (decomp.), slightly soluble in water, but insoluble in alcohol or ether (Found: C, 35·6; H, 5·9%; equiv., 204. $C_6H_{12}O_4N_4$ requires C, 35·3; H, 5·9%; equiv., 204). Determination of formaldehyde by the direct Romijn titration gave low and variable results; it appears that in this case the liberation of formaldehyde and its reaction with iodine do not proceed smoothly to completion in alkaline solution, the best figures (13·7, 13·9%; cf. theoretical 14·7%) being obtained when the aqueous solutions were kept for 35 and 50 minutes, respectively, before proceeding with the determination. Attempts to form the morpholine salt of nitrourea by mixing the anhydrous constituents resulted in immediate decomposition, and evaporation, *in vacuo*, of an aqueous solution of the salt yielded morpholine-4-carboxyamide (Knorr, *Annalen*, 1898, **301**, 8; for the course of reaction see Davis and Blanchard, *J. Amer. Chem. Soc.*, 1929, **51**, 1790).

4-2′-*Nitroisobutylmorpholine* (VII).—2-Nitropropane (9 ml.), morpholine (10 ml.), 38% formaldehyde (10 ml.), and 10N-sodium hydroxide (0·2 ml.) were warmed to 30°, and sufficient alcohol was added to give a homogeneous solution. After 15 hours at 30° the mixture was acidified, diluted with water (20 ml.), and extracted with ether. The extract was washed with sodium hydrogen carbonate, dried, and distilled. 4-2′-Nitro*iso*butylmorpholine (8·5 g.), b. p. 106—108°/2 mm., crystallised from ligroin (b. p. 60—80°) in stout prisms or cubes, m. p. 46—48° (Found: C, 51·0; H, 8·4; N, 14·9. Calc. for $C_8H_{16}O_3N_2$: C, 51·0; H, 8·6; N, 14·9%), soluble in organic solvents, but insoluble in water; the solution in aqueous alcohol could not be titrated with alkali. Johnson (*J. Amer. Chem. Soc.*, 1946, **68**, 13) has since reported m. p. 43°, b. p. 90—91°/0·5 mm.

Apparent Molecular Weight of N-*Morpholinomethylmethylnitroamine.*—Concentration in water, 3·98, 5·47, 5·92 g./l.: apparent *M*, 57, 64, and 62, respectively.

Concentration in 0·967N-hydrochloric acid, 6·32, 9·48, 9·66, 20·24 g./l.: apparent *M*, 500, 470, 530, and 800, respectively.

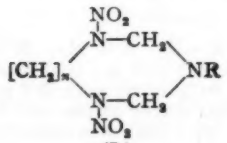
THE UNIVERSITY, SHEFFIELD, 10. [*Received, December 15th*, 1948.]

356. *Studies on Nitroamines. Part VI. The Nitration of some Aminomethylnitroamines.*

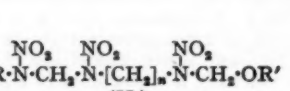
By F. Chapman, P. G. Owston, and D. Woodcock.

The compounds (I; $n = 1$ or 2; R = alkyl) and (III)—(VI), prepared as described in Part IV of this series, have been nitrated at various temperatures with combinations of 98% nitric acid, acetic acid, acetic anhydride, and ammonium nitrate, and the main reaction products identified.

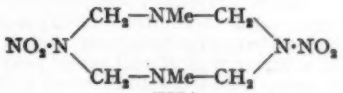
1 : 5-Dinitro-3-alkylhexahydro-1 : 3 : 5-triazines (I; $n = 1$; R = Me or Et), on treatment with 98% nitric acid at 10°, gave the corresponding linear nitrate esters (II; R = Me or Et; R′ = NO_2), whilst treatment with nitric acid at 50—60° in the presence of acetic acid, acetic anhydride, and ammonium nitrate resulted in the formation of the acetates (II; R = Me or Et; R′ = Ac). These results confirm the structures previously assigned (Part IV) to the initial materials. Nitration of the 3-methyl compound (I; $n = 1$; R = Me) with a mixture of 98% nitric acid and ammonium nitrate at 60—65° or 95—100° gave cyclonite (I; $n = 1$; R = NO_2) as the only identifiable product. A very small amount of cyclonite was also obtained by nitration of the 3-ethyl compound (I; $n = 1$; R = Et) with 98% nitric acid, acetic acid, acetic anhydride, and ammonium nitrate at 55—60°.



(I.)



(II.)

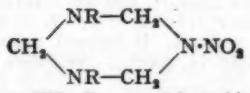


(III.)

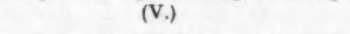
The structure of the *nitrate* (II; $n = 1$; R = Et; R′ = NO_2) was confirmed by conversion into the corresponding methyl ether and acetate (R′ = Me or Ac) by boiling methyl alcohol or sodium acetate–acetic acid, respectively.

The 1 : 5-dinitro-3-alkyl-1 : 3 : 5-triaza*cyclo*heptanes (I; $n = 2$; R = Me, Et, Pr^i, Bu^n, or *cyclo*hexyl) were nitrated with acetic anhydride and 98% nitric acid at 55° in the presence or absence of ammonium nitrate. Without ammonium nitrate the corresponding linear *compounds* (II; $n = 2$; R = Me, etc.; R′ = Ac) were invariably formed. With ammonium nitrate, compounds of type (II; $n = 2$) may still be the only products which can be isolated, *e.g.*, from (I; $n = 2$; R = Me or Bu^n) the homologue of cyclonite (I; $n = 2$; R = NO_2) may be formed simultaneously, *e.g.*, from (I; $n = 2$; R = Et), or it may be the only product of nitration which can be isolated, *e.g.*, from (I; $n = 2$; R = Pr^i or *cyclo*hexyl).

From these results it seems that compounds of type (I; $n = 1$ or 2; R = alkyl) can readily undergo nitration to give linear compounds (II; $n = 1$ or 2) or ring compounds (I; $n = 1$ or 2; R = NO_2). The evidence available is not sufficient to enable the reaction products to be predicted, but it is noteworthy that cyclonite and its homologue have been isolated only when the nitration mixture contained ammonium nitrate.



(IV; R = *cyclo*hexyl.)



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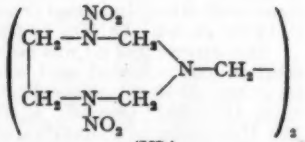


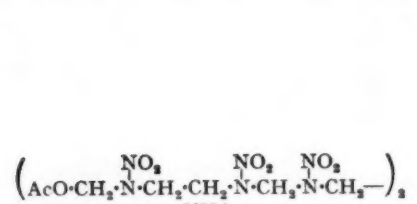
(VI.)

No identifiable product could be obtained by treating the cyclic compound (III) (Chute, Downing, McKay, Myers, and Wright, " Nitrolysis of Hexamine, Part I," *Canadian J. Res.*, in the press) with 98% nitric acid at 10°, but, by use of a mixture of 98% nitric acid, acetic anhydride, and ammonium nitrate at 55—60°, an excellent yield of the acetate (II; $n = 1$; R = Me; R′ = Ac) was obtained.

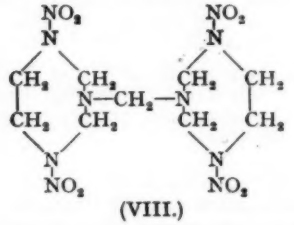
cyclo*Hexylamine nitrate* was the only compound which could be isolated by nitration of (IV) with 98% nitric acid at 10°. 98% Nitric acid, acetic acid, acetic anhydride, and ammonium nitrate at 55—60° gave a 15% yield of cyclonite.

The acetate (II; $n = 1$; R = Me; R′ = Ac) was obtained by nitration of (V) with 98% nitric acid, acetic acid, acetic anhydride, and ammonium nitrate at 50—60°.

The nitration product obtained by treatment of 3 : 3′-ethylenebis-(1 : 5-dinitro-1 : 3 : 5 triaza*cyclo*heptane) (VI) with 98% nitric acid and acetic anhydride was the same, whether or



(VII.)



(VIII.)

not ammonium nitrate was added. Analyses gave results in good agreement with those required for *compound* (VII), as expected by analogy with the behaviour of compounds of type (I; $n = 1$ or 2; R = alkyl) under similar conditions. It is, however, interesting to contrast the behaviour of the compound (VI) with that reported for the corresponding methylene compound (VIII) (Myers and Wright, " Nitrolysis of Hexamine, Part IV," *Canadian J. Res.*, in the press), which on comparable nitration gave a mixture of the linear compound (II; $n = 2$; $R = CH_2{\cdot}OAc$; R′ = Ac) and the cyclic compound (I; $n = 2$; $R = NO_2$).

Experimental.

NN′N″-*Trinitro*(*acetoxymethylaminomethyl*)(*methylaminomethyl*)*amine* (II; $n = 1$; R = Me; R′ = Ac).—(*a*) 1 : 5-Dinitro-3-methylhexahydro-1 : 3 : 5-triazine (I; $n = 1$; R = Me) (1·9 g., 0·01 mol.) was added, simultaneously with a solution of ammonium nitrate (2·4 g.) in 98% nitric acid (3·2 g.), to a mixture of glacial acetic acid (6·9 c.c.) and acetic anhydride (6·5 c.c.) and stirred at 50—60° for ¾ hour. After the addition of water (*ca.* 40 c.c.), a white solid (2·2 g.), m. p. 140—146°, was collected, washed with water, and dried.

(*b*) 1 : 5-Dinitro-3 : 7-dimethyl-1 : 3 : 5 : 7-tetra-aza*cyclo*octane (III) (1·7 g., 0·007 mol.) was treated in the same manner with the appropriately reduced quantities of the other reagents; yield, 3·7 g.; m. p. 134—136°.

(*c*) Methyldi-(*N*-nitro-*N*-methylaminomethyl)amine (V) (1·8 g., 0·009 mol.) was treated similarly with acetic anhydride (4·5 c.c.) and the appropriately reduced quantities of the other reagents; yield, 1·0 g.; m. p. 114—125°.

Crystallisation of each crude product from glacial acetic acid gave rosettes of prisms, m. p. 153—154°, which were shown to be (II; $n = 1$; R = Me; R′ = Ac) (Canadian workers, private communications) by analyses, determinations of mixed m. p., and a comparison of *X*-ray diffraction diagrams.

Treatment of 1 : 5-*Dinitro*-3-*methylhexahydro*-1 : 3 : 5-*triazine* (I; $n = 1$; R = Me) *with Nitric Acid Alone.*—The compound (1·4 g.) was stirred with 98% nitric acid (12 c.c.) for 0·5 hour and poured into dry ether (50 c.c.). The crude gum hardened on trituration with further portions of dry ether, and was converted into (II; $n = 1$; R = Me; R′ = Et) by boiling with fused sodium acetate in acetic acid, cooling, and dilution with water, or into the methyl ether (II; $n = 1$; R = R′ = Me), plates, m. p. 110° [workers at The University of Pennsylvania (private communications) give m. p. 114°], by heating under reflux with excess of methyl alcohol for 2 hours and cooling.

NN′N″-*Trinitro*(*nitroxymethylaminomethyl*)(*ethylaminomethyl*)*amine* (II; $n = 1$; R = Et; R′ = NO_2).—1 : 5-Dinitro-3-ethylhexahydro-1 : 3 : 5-triazine (I; $n = 1$; R = Et) (1·0 g.) was added to 98% nitric acid (10 c.c.), stirred at 10—15° for ½ hour, and then added dropwise to dry ether (50 c.c.) at <15°. The white crystalline *nitrate* (0·91 g.), m. p. 111—114° (decomp.), was collected, washed three times with ether, and dried [Found: C, 19·1; H, 4·4; $O{\cdot}NO_2$ (nitron), 20·75, 20·82. $C_5H_{11}O_9N_7$ requires C, 19·2; H, 3·5; $O{\cdot}NO_2$, 19·8%].

This nitrate (0·2 g.) was boiled under reflux with methyl alcohol (4 c.c.) for 2 hours, and the resultant solution concentrated and allowed to cool. The crystalline methyl ether (II; $n = 1$; R = Et; R′ = Me) (0·10 g.) was collected, dried, and recrystallised from methyl alcohol; it formed prisms, m. p. 101—102° (Found: C, 25·8; H, 5·0. Calc. for $C_6H_{14}O_7N_6$: C, 25·5; H, 5·0%). Workers at The University of Pennsylvania (private communication) give m. p. 96°.

NN′N″-*Trinitro*(*acetoxymethylaminomethyl*)(*ethylaminomethyl*)*amine* (II; $n = 1$; R = Et; R′ = Ac).—(*a*) 1 : 5-Dinitro-3-ethylhexahydro-1 : 3 : 5-triazine (I; $n = 1$; R = Et) (1·0 g.) was added, simultaneously with a solution of ammonium nitrate (1·2 g.) in 98% nitric acid (1·1 c.c.), to a mixture of glacial acetic acid (3·5 c.c.) and acetic anhydride (3·3 c.c.) stirred at 55—60°. Solid commenced to separate after the addition was complete, and stirring was continued for ½ hour, whereafter water (30 c.c.) was added. The product (0·96 g.), m. p. 114—116°, was collected, washed with water, and dried in a desiccator. Repeated crystallisation from methyl alcohol gave monoclinic prisms, m. p. 132° (workers at The University of Pennsylvania, private communication, give m. p. 133—134°). Cyclonite, m. p. 200—201° (decomp.), was isolated in minute amount from a fraction less soluble in methyl alcohol.

(*b*) The nitrate (II; $n = 1$; R = Et; R′ = NO_2) (0·1 g.) was boiled under reflux for 2 minutes with fused sodium acetate (0·1 g.) in glacial acetic acid (1 c.c.). The solution was cooled, water (1 c.c.) added, and the crystalline precipitate was collected, washed with water, and dried in a vacuum; yield, 0·07 g.; m. p. 131—132°, unaltered by recrystallisation from methyl alcohol.

Nitration of 1-*Nitro*-3 : 5-*dicyclohexylhexahydro*-1 : 3 : 5-*triazine* (IV).—(*a*) *By* 98% *nitric acid.* The compound (1·0 g.) was added to 98% nitric acid (10 c.c.) and stirred at 10—15° for ½ hour and then added dropwise, with stirring, to dry ether (60 c.c.) at <15°. The gum which separated was washed by stirring with fresh ether and dissolved in methyl alcohol; the solution was concentrated and kept at 0° overnight. Recrystallisation, from alcohol–ether, of the precipitate thus obtained, gave *cyclohexylamine nitrate* as prisms, m. p. 153—154° (decomp.) (Found: C, 43·4; H, 8·4; N, 17·6; O·NO_2 (nitron), 37·4. $C_6H_{14}O_3N_2$ requires C, 44·5; H, 8·6; N, 17·3; O·NO_2, 38·2%). The product was very soluble in water and gave a positive brown-ring test for nitrates, and the odour of *cyclo*hexylamine was observed after warming it with dilute sodium hydroxide.

(*b*) The compound (IV) was heated at 50—60° for ½ hour with ammonium nitrate, nitric acid, acetic acid, and acetic anhydride. The sticky solid precipitated by water yielded cyclonite on crystallisation from acetone.

Nitration of 1 : 5-*Dinitro*-3-*alkyl*-1 : 3 : 5-*triazacycloheptanes* (I; $n = 2$; R = Me, Et, etc.).—(*a*) *By* 98% *nitric acid and acetic anhydride at* 55°. The *cyclo*heptane (1·0 g.) was dissolved in acetic anhydride (10 c.c.) and warmed to 55° with stirring. 98% Nitric acid (4 c.c.) was then added at such a rate that the temperature remained constant at 55° ± 2°. After 15 minutes, the solution was diluted with water (40 c.c.); the linear nitration product (II; $n = 2$; R′ = Ac; R = Me, Et, etc.) was obtained as a sticky gum, which could be crystallised from methyl alcohol or a mixture of methyl alcohol, acetone, and water. Details of the acetates (II; $n = 2$; R′ = Ac; R = Me, etc.) and the yields obtained from 1 g. of the corresponding compound (I; $n = 2$) are recorded in Table I.

TABLE I.

Compounds, $NO_2{\cdot}NR{\cdot}CH_2{\cdot}N(NO_2){\cdot}CH_2{\cdot}CH_2{\cdot}N(NO_2){\cdot}CH_2{\cdot}OAc$.

R.	Crystal form, and solvent.	M. p	Analysis.	Yield.
Me	Clusters of prisms; acetone–methanol	89—91°	Found: C, 27·4; H, 4·5; N, 26·8%. $C_7H_{14}O_8N_6$ requires C, 27·1; H, 4·5; N, 27·1%	0·5 g.
Et	Prisms; acetone–methanol–water	64—67	Found: C, 30·0; H, 5·0; N, 25·3%. $C_8H_{16}O_8N_6$ requires C, 29·6; H, 4·9; N, 25·9%	1·0
Pr^i	Clusters of prisms; acetone–methanol–water	69—72	Found: C, 31·9; H, 5·6; N, 23·6%. $C_9H_{18}O_8N_6$ requires C, 32·0; H, 5·3; N, 24·9%	0·7
Bu^n	Prisms; methanol–water	92—94	Found: C, 33·6; H, 5·9; N, 24·2%. $C_{10}H_{20}O_8N_6$ requires C, 34·1; H, 5·7; N, 23·9%	0·95
*cyclo*Hexyl	Prisms; acetone–methanol–water	63—65	Found: C, 37·8; H, 5·8; N, 22·3%. $C_{13}H_{22}O_8N_6$ requires C, 38·1; H, 5·8; N, 22·2%	0·95

(*b*) *By* 98% *nitric acid, acetic anhydride, and ammonium nitrate at* 55°. The nitrations were carried out as in (*a*), except that a solution of ammonium nitrate (1·7 g.) in 98% nitric acid (2·3 g.) was added instead of pure 98% nitric acid. Details of the nitration products obtained in each case are recorded in Table II, the identities being established by mixed melting points.

TABLE II.

Compound nitrated. (I; $n = 2$), R =	Nitration product(s).	Yield (from 1 g.).
Me	(II; $n = 2$; R = Me; R′ = Ac)	0·3 g.
Et	(II; $n = 2$; R = Et; R′ = Ac)	0·6
	(I; $n = 2$; R = NO_2)	0·2
Pr^i	(I; $n = 2$; R = NO_2)	0·6
Bu^n	(II; $n = 2$; R = Bu^n; R′ = Ac)	0·7
*cyclo*Hexyl	(I; $n = 2$; R = NO_2)	0·4

Nitration of 3 : 3′-*Ethylenebis*-(1 : 5-*dinitro*-1 : 3 : 5-*triazacycloheptane*) (VI) *by* 98% *Nitric Acid and Acetic Anhydride.*—The compound (VI) (1 g.) was dissolved in acetic anhydride (150 c.c.) at 55° with stirring. 98% Nitric acid (25 c.c.) was added dropwise, and the temperature maintained at 55°. When the addition of the acid was complete, the *diacetate* (VII) began to separate. The nitration mixture was cooled and the solid collected. By repeated recrystallisation from glacial acetic acid, the product (0·85 g.) was obtained as rosettes of prisms, m. p. 161—162° [Found: C, 27·3; H, 4·1; N, 26·6%; *M*, (Rast), 561, 572; acetyl, 15·1. $C_{14}H_{26}O_{16}N_{12}$ requires C, 27·2; H, 4·2; N, 27·2; *M*, 618; acetyl, 13·9%).

The authors wish to thank Dr. E. Roberts for a pure specimen of the acetate (II; $n = 1$; R = Et; R′ = Ac), and Professor F. J. Llewellyn for determining the *X*-ray diffraction diagrams.

THE UNIVERSITY, SHEFFIELD, 10. [*Received, December 15th*, 1948.]

357. *Studies on Nitroamines. Part VII. The Decomposition of Methylenedinitroamine in Aqueous Solutions.*

By Alex. H. Lamberton, C. Lindley, and J. C. Speakman.

Methylenedinitroamine decomposes in 1N-mineral acid, in 2N-sodium hydroxide, and at pH in the range 3—8. Considerable stability is exhibited around pH 1 and pH 10.

The decomposition at pH 3—8 leads finally to nitrous oxide and formaldehyde. The primary stage appears to result in the liberation of nitroamine and the conversion of the methylene group into a form oxidisable by alkaline hypoiodite. The decomposition is autocatalytic and is catalysed, though less markedly, by extraneous formaldehyde. It is suggested that the mono-ion shows inherent instability, and the kinetics of the reaction are discussed.

A method of determination has been elaborated which is applicable in the presence of unknown quantities of acid and formaldehyde.

The investigation of the stability of methylenedinitroamine was undertaken originally on account of the possible role of this compound in the synthetic production of cyclonite. Since, however, information has been gained regarding the effect of one nitroamino-substituent on a similar group situated in the α-position to the first, the results are of general interest in the field of nitroamine chemistry.

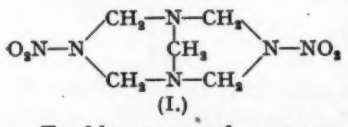
On heating in water, methylenedinitroamine has been shown (Roberts, private communication) to yield, finally, formaldehyde and nitrous oxide, thus:

$$CH_2(NH{\cdot}NO_2)_2 \longrightarrow CH_2O + 2N_2O + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The quantitative production of formaldehyde and nitrous oxide has been confirmed, and preliminary studies were carried out mainly by the measurement of the evolution of gas from heated solutions. However, this method, though convenient and rapid, does not necessarily provide a true measure of the primary stage of decomposition, since products intermediate between the dissolved nitroamine and the evolved gas are conceivable. Supersaturation by gas affects the results in the same sense. In fact (see below), methylenedinitroamine is somewhat, though not markedly, less stable than the results of gas evolution indicate. It has been assumed, by analogy with the behaviour of other primary nitroamines, that complete decomposition in strong mineral acid would give rise to two molecular proportions of gas. A similar volume is produced on heating with aqueous sodium hydroxide, though unpublished work indicates that this is a mixture of nitrogen and nitrous oxide.

Decomposition, as measured by gas evolution, occurred with moderate rapidity (50% in about 1 hour at 70°) on warming in strong (11N.) nitric or sulphuric acid. At lower acid concentrations (N. and 0·1N.), stability was great and appeared, by comparison with trimethylenedinitroamine, to be greater than that shown by an isolated primary nitroamino-group. Tests with phosphate buffers showed that, in contrast with trimethylenedinitroamine, a zone of rapid decomposition (50% in a few minutes at 70°) extended from pH 3 to pH 8; this was succeeded by a second region of stability around pH 10, whilst rapid decomposition was observed again in 2N-sodium hydroxide. These results are presented in Table I (Experimental; section *C*).

The precipitation of 3 : 7-dinitro-1 : 3 : 5 : 7-tetra-aza*bicyclo*[3 : 3 : 1]nonane (I) from solutions of monoammonium methylenedinitroamine in aqueous formaldehyde suggested the formation of nitroamine as an intermediate, and the decomposition in water was subjected to a more detailed investigation by acidimetry and the iodometric determination of available formaldehyde.

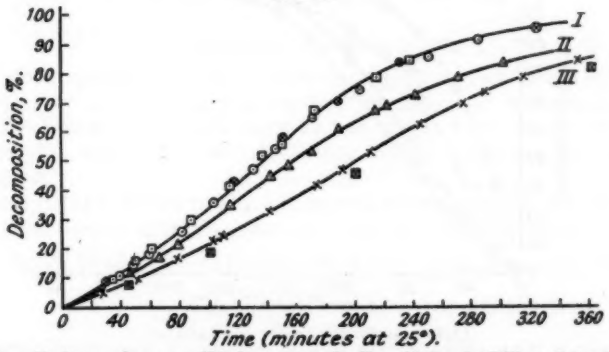


(I.)

Freshly prepared aqueous solutions of methylenedinitroamine could be titrated, using thymolphthalein, to a sharp end-point. As decomposition proceeded, there was a decrease in the volume of sodium hydroxide required to effect neutralisation, but the residual acidity thus determined was not an accurate measure of the methylenedinitroamine content of the solutions. The end-point remained sharp, but thereafter, on standing, the solutions became more alkaline, and the colour of the indicator could no longer be removed by the addition of one drop of 0·1N-hydrochloric acid. In less than 25 minutes, stability was re-established, and the alkali which had been set free could be determined by back-titration with hydrochloric acid. It was thus found that part of the "total acidity" originally determined by direct titration with sodium hydroxide was destroyed on being left for 25 minutes under mildly alkaline conditions; the rest, by contrast, could be termed "permanent." Methylenedinitroamine was shown to be unaffected by the degree of alkalinity employed

(Experimental; section *F*), and the extent of the decomposition calculated from the " permanent acidity " agreed with that found by iodometric determination of available formaldehyde (Experimental; section *D*). At 70°, measurements of " permanent acidity " and formaldehyde (iodometrically) were again consistent, and indicated a greater degree of decomposition than those of " total acidity," gas evolution, or formaldehyde by precipitation as the 2 : 4-dinitrophenylhydrazone (Experimental; section *E*). These phenomena are explicable in terms of the known properties of nitroamine and hydroxymethylnitroamine (Part V, this series). We

FIG. 1.

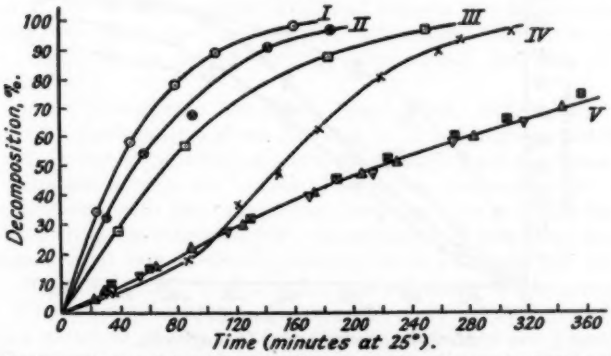


Methylenedinitroamine : M./20 *in acetate buffers* (2·5M-AcOH + 2·5M-NaOH).

I. { *pH* 5·15 ⟶ 5·25 ⊙. *pH* 5·25 ⟶ 5·35 ⊗. *pH* 5·45 ⟶ 5·60 ⊡.
II. *pH* 5·80 ⟶ 6·20 △.
III. { *pH* 4·65 ⟶ 4·70 ×. *pH* 4·60 ⟶ 4·70 *in half-strength buffer* ⊠.

(*pH values to nearest* 0·05 *unit.*)

FIG. 2.



Methylenedinitroamine at pH 4·65 *and* 5·35 : *variations in concentration and solvent.*

(*Acetate buffers,* 2·5M., *unless specified.*)

pH 4·65 { *I.* M./20 + 0·195M-CH_2O ⊙. *II.* M./5 + 0·195M-CH_2O ⊗. *III.* M./20 + 0·045M-CH_2O ⊡. *IV.* M./5 ——— ×.

pH 5·35, *V.* { M./100 ——— △. M./100 *in half-strength buffer* ⊠. M./100 *in* 2M-*phosphite buffer* ▽.

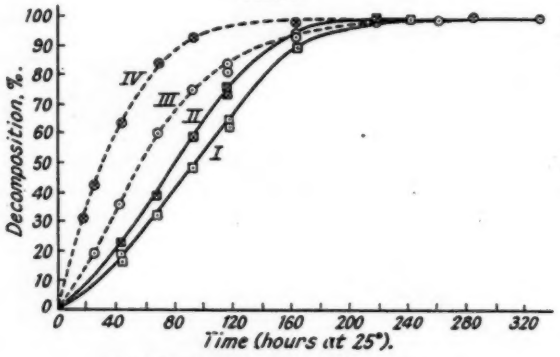
(*pH values to nearest* 0·05 *unit.*)

therefore believe that the first stage of the decomposition in aqueous solution (and by analogy in buffers at pH 3—8) results in the production of nitroamine and/or a simple derivative of nitroamine; the methylene group is also rendered accessible to oxidation by iodine in alkaline solution, thus providing a convenient measure of the extent of decomposition.

In the presence of unknown quantities of acid and formaldehyde, methylenedinitroamine may be determined by precipitation of the barium salt, but this salt is unstable, decomposing with loss of weight on prolonged drying in a vacuum; furthermore, co-precipitation cannot be detected. It is, therefore, preferable to decompose the barium salt and determine the liberated formaldehyde. The method is of diagnostic value (see Part IV).

Factors influencing the Rate of Decomposition at 25°.—(i) *pH of the solution.* The results supported the general conclusions derived from measurements of gas evolution at 70°. Stabilisation by mineral acid or extremely dilute alkali was confirmed (Experimental; section *F*). In acetate buffers the rates of decomposition at pH 5·2, 5·3, and 5·5 were experimentally indistinguishable, and were greater than those observed at pH 4·7 or 6·0; the maximum is thus in the region of pH 5·4 (Fig. 1).

FIG. 3.



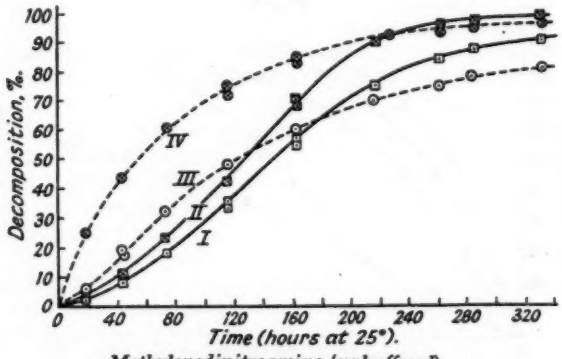
Methylenedinitroamine (unbuffered).

M./20-*solutions.*

M./20 *in water :* ———— *Curve I, by " total acidity,"* ⊡. *Curve II, by iodometric* CH_2O ⊠.

M./20 *in* 0·0530M-CH_2O : – – – – – – *Curve III, by " total acidity,"* ⊙. *Curve IV, by iodometric* CH_2O ⊗.

FIG. 4.



Methylenedinitroamine (unbuffered).

M./5-*solutions.*

M./5 *in water :* ———— *Curve I, by " total acidity "* ⊡. *Curve II, by iodometric* CH_2O ⊠.

M./15 *in* 0·2025M-CH_2O : – – – – – – *Curve III, by " total acidity "* ⊙. *Curve IV, by iodometric* CH_2O ⊛.

(ii) *Concentration of methylenedinitroamine.* In acetate buffers at a given pH, the initial percentage rates of decomposition were similar for M/5-, M/20-, and M/100-solutions; the more concentrated the solution, the more rapid was the subsequent development of the decomposition (compare curves I and III of Fig. 1 with curves V and IV, respectively, of Fig. 2). In water a M/5-solution decomposed more slowly than did a M/20-solution; this may be due to its enhanced acidity.

(iii) *Catalysis.* The S-shape of the curves suggested that the reaction was catalytic, and formaldehyde was found to accelerate markedly the decomposition in a buffer of pH 4·7 (compare curves I and III of Fig. 2 with curve III of Fig. 1; also compare curves II and IV of Fig. 2), in water (Figs. 3 and 4, compare curves II and II with IV and IV), and in 0·1N-hydrochloric acid (Table IV). As expected, the effect was most marked at the start of the reaction. In most cases, additional self-catalysis could be shown by analysis (see General Discussion

below); but complexities were clearly indicated by the results for M/5-solutions in water and in M/5-formaldehyde; the initially-greater rate of decomposition in formaldehyde was later surpassed by that of the powerfully self-catalysed decomposition in water (Fig. 4, curves II and IV).

In the case of M/100-solutions at pH 5·35, dilution of the acetate buffer with an equal volume of water, or its replacement by a phosphate buffer, had little effect on the course of decomposition (Fig. 2, curve V). The slight decrease in rate on dilution of the buffer with water in the case of an M/20-solution at pH 4·7 may be due simply to the enhanced drift in pH arising from the lowered buffering capacity (Fig. 1, curve III).

General Discussion.

Kinetics.—The decomposition of methylenedinitroamine does not appear to follow any simple kinetic course, but our experiments warrant the following comments.

(*a*) The generally S-shaped reaction–time curves (Figs. 1, 2, 3, and 4) suggest that the reaction is autocatalytic, and the finite initial rate further suggests that there must also be a spontaneous reaction.

(*b*) If (i) the catalytically active product is formaldehyde and (ii) the spontaneous reaction is of the first order, the overall kinetics should be expressed by the equation :

$$-\mathrm{d}[\mathrm{CH_2(NH{\cdot}NO_2)_2}]/\mathrm{d}t = k_s[\mathrm{CH_2(NH{\cdot}NO_2)_2}] + k_c[\mathrm{CH_2(NH{\cdot}NO_2)_2}][\mathrm{CH_2O}]$$

$$i.e., \quad \frac{-\mathrm{d}[\mathrm{CH_2(NH{\cdot}NO_2)_2}]/\mathrm{d}t}{[\mathrm{CH_2(NH{\cdot}NO_2)_2}]} = k_o = k_s + k_c[\mathrm{CH_2O}] \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

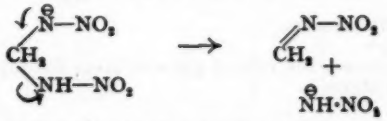
k_s and k_c being the specific rate constants of the spontaneous and autocatalysed reactions, and k_o the apparent, instantaneous, first-order constant obtainable at any stage from the slope of the reaction–time curve. According to equation 2, a plot of k_o against formaldehyde concentration should give a straight line which intersects the axis at k_s and has a slope of k_c. Within experimental error, this relation appears to hold for the experiments on the decomposition of methylenedinitroamine in water or in buffer solutions at pH 4·7 and 5·2.

(*c*) However, the reaction is more complex than this, for, according to equation 2, results for M/20-, M/5-, and M/100-methylenedinitroamine at a given pH should yield points lying on the same straight line, whereas in fact they do not. Moreover, when extraneous formaldehyde is present, the decomposition is indeed initially accelerated, but not to the extent to be anticipated from equation 2.

(*d*) From the known dissociation constants (Part VIII, this series), the proportion of methylenedinitroamine mono-ion at a given pH and infinite dilution could be calculated. If it is borne in mind that, in the application of these results to actual solutions, no correction was made for salt-concentration effects, the values of k_s and k_c [subsection (*b*)] at pH 4·7 and 5·2 and in water are consistent with the suggestion that the rates of both the spontaneous and autocatalytic reactions are proportional to the concentration of the mono-ion; and the observation that maximum overall rate of decomposition occurs at about pH 5·4 is consistent with the (uncorrected) calculation that the maximum concentration of mono-ion occurs at pH 5·8.

Applications to Nitroamine Chemistry.—Though the observed stability of the un-ionised and of the doubly ionised material at pH 1 and 9, respectively, does not guarantee their stability at intermediate pH values, the hypothesis that decomposition proceeds specifically through the mono-ion is favoured (preceding section).

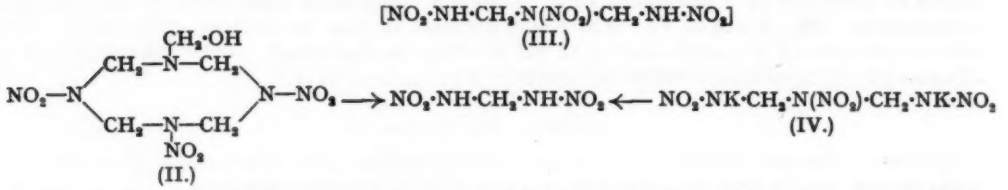
It may be that, in the spontaneous reaction, one nitroamino-group cannot facilitate the ejection of the other as a nitroamine anion; but, after mono-ionisation has taken place, the relatively greater availability of electrons permits this rupture, thus :



In the doubly ionised material there is electron availability from both nitroamino-nitrogen atoms, but there remains no relatively attractive grouping to promote migration. It is not considered that our present knowledge permits the formulation of any mechanism for the catalytic reaction.

Whatever theories of decomposition may be advanced, it appears that the system $NO_2{\cdot}NR{\cdot}CH_2{\cdot}\overset{\ominus}{N}{\cdot}NO_2$ is unstable in aqueous solution, and there is no obvious reason to limit

this instability to the case of R = H. Furthermore, when R is not ionisable, the possibility of stabilisation in alkali by formation of the di-ion is removed. This may explain why, in two cases, methylenedinitroamine has been isolated in place of a higher nitroamino-methylene homologue. For example, workers at Bristol (private communication) prepared a compound which they believed to be (II), by nitrolysis of (I). It yielded nitrous oxide and methylene-



dinitroamine, and not (III), on treatment with alkali. Again, Canadian workers (private communication) have prepared the compound (IV) which, though formally a salt of (III), yielded methylenedinitroamine on neutralisation with acid.

Experimental.

A. General.—Determinations of formaldehyde were made iodometrically unless otherwise stated. In acidimetry a methyl alcoholic solution of thymolphthalein was used as indicator. The methylenedinitroamine (Part II, this series) was recrystallised from ether. pH values were measured with the glass electrode; those at 70° are derived by an admittedly speculative extrapolation from data at 18° and 40°, but are held to be correct to within the limits stated. The pH ranges quoted indicate the probable change during the decomposition of the methylenedinitroamine.

B. Total Gas Evolution from Methylenedinitroamine.—0·1M-, 0·05M-, and 0·025M-aqueous methylenedinitroamine evolved 1·96, 1·97, and 2·08 molecular proportions of gas on maintenance at 70° in a closed system. 0·05M-Solutions in 2N-sodium hydroxide evolved 1·96 and 1·95 molecular proportions under the same conditions.

C. Measurement of the Rate of Gas Evolution.—Consistent results could not be obtained by the addition of solid methylenedinitroamine to preheated buffer solutions in a closed system. The presumed supersaturation was overcome, and a reasonable agreement between individual experiments obtained, by the following technique.

Table I.

(All solutions initially M./20 with respect to dinitroamine.)

Solvent.	pH during decomposition (estimated at 70°).	Time, in minutes, for 50% decomposition (by gas evolution) on rapid heating (20° → 70° in 5 minutes) and maintaining at 70°. Methylenedinitroamine.	Trimethylenedinitroamine.
11·2N-HNO_3		70 *	4·5
10·8N-H_2SO_4		110	5
1·0N-HNO_3		>1500 †	120
2·0M-H_2PO_4		>1000 †	180
0·1N-HNO_3		> 500 †	> 500 †
Phosphate buffers: 2·0M-H_2PO_4 2·0N-NaOH	2	60	>1000 †
	3	8	
	4·7 → 5·2	2·5	
	5·6 → 5·9	3	stable
	6·4 → 6·6	6	
	7·5 → 7·8 ‡	50	
	10·0	> 500 †	
	11·0 11·6	130	
2·0N-NaOH		4·5	

* Fume-off occurred at *ca.* 70 minutes.

† Estimated by extrapolation.

‡ pH 7·8 at 50% decomposition; the rate of gas evolution fell rapidly thereafter as, with lowered buffering capacity, the pH rose above 8.

The reaction vessel (a tube of *ca.* 20 ml. capacity, with a tap, giving access to the air, sealed to a side arm) was loosely filled with glass wool. A 50-ml. dropping funnel, the stem of which was jacketed to form a small condenser, was attached, and the reaction vessel, with its tap open to the air, was immersed in the thermostat. The freshly prepared solution under investigation was introduced into the dropping funnel, and connection made from the mouth of the dropping funnel to the nitrometer. With the nitrometer-tap partly removed to give access to the air, the solution was run into the reaction vessel, and the clock started. After 15 seconds, drainage was expressed from the dropping funnel by manipulation of the rubber tube leading to the nitrometer; after 30 seconds, the tap of the reaction

vessel was closed and the nitrometer tap inserted in position. The liquid thus introduced was found to rise in temperature to approximately 45° after 1 minute, 60° after 2 minutes, and 70° after 5 minutes. In blank experiments a consistent increase in gaseous volume was observed after closing the tap. The crude volumes of gas evolved were appropriately corrected. Results are recorded in Table I.

D. Measurement of the Degree of Decomposition; Relation between Available Formaldehyde, Total Acidity, and Permanent Acidity for Methylenedinitroamine Solutions in Water or Dilute Formaldehyde.—Solutions of methylenedinitroamine were maintained at 25°, and the apparent percentage decomposition measured by the following methods. (*a*) "Total acidity": an aliquot was titrated directly with standard sodium hydroxide; time required for titration about 2 minutes. (*a'*) "Permanent acidity": the titrated aliquot from (*a*) was set aside for 25 minutes at room temperature, and then back-titrated with standard hydrochloric acid. The "permanent acidity" was found by subtracting the titre of hydrochloric acid from that of sodium hydroxide required in operation (*a*). (*b*) "Formaldehyde without destruction of nitroamine": the titrated aliquot from (*a*) was determined immediately [a treatment alternative to (*a'*)]. (*b'*) "Formaldehyde after destruction of nitroamine": the back-titrated aliquot from (*a'*) was analysed. For results see Table II.

TABLE II.

Concentration; solvent; run no.	Time (hours) at 25°.	Apparent % decomposition by method: (*a*.)	(*a'*.)	(*b*.)	(*b'*.)
M./20 in water; I	0	1	1	—	2
	49	21, 21	27	29	—
	51	23, 21	28	29	30
M./20 in 0·0527M-formaldehyde; II ...	0	0	0	—	2
	47	41, 41	69	70	69
	50	43	72	—	73

E. Relation between Available Formaldehyde, Acidity, and Gas Evolution for Solutions of Methylenedinitroamine in Water at 70°.—To methylenedinitroamine was added sufficient distilled water, at 70°, to give a M/20-solution which was maintained at 70° in a thermostat. Aliquots were run into ice, and thereafter determined, as in section *D*, by methods *a*, *a'*, *b*, and *b'*; formaldehyde was also determined by means of Brady's reagent. The gas-evolution results (admittedly less accurate) were obtained graphically from separate experiments in which methylenedinitroamine was added to water, at 70°, in a closed system. Results are recorded in Table III.

TABLE III.

Run nos.	Time (minutes) at 70°.	Apparent % decomposition. Method (see Section *D*). (*a*.)	(*a'*.)	(*b*.)	(*b'*.)	Brady.	Gas.
III & IV	5	5	—	18	—	—	2
	10	16	29	—	32	21	10
	15	29	—	47	—	—	25
	20	42	59	—	61	46	39
	22	45	—	65	—	—	45
	27	58	—	78	—	—	58
	30	66	83	—	85	67	65
	33	72	—	91	—	—	72
	43	88	—	100	—	—	87
	63	98	—	102	—	—	95
	85	100	—	101	—	—	96

F. Stabilisation by Acid and by Alkali.—(*a*) Solutions of methylenedinitroamine (M/20 in N/10-hydrochloric acid) were maintained at 25°, and the apparent percentage decomposition (see Table IV) measured by methods (*a*) and (*b*) (section *D*).

TABLE IV.

Concentration; solvent.	Run no.	Method of determination.	Apparent % decomposition. Hours at 25°. 19·5	116	265	407	524	*ca.* 600; then heated for 45 min. at 80° with buffer of pH 5·2.
M./20 in M/10-HCl	V	(*a*)	1	1	2	3	4	
		(*b*)	0	1	3	4	6	100
M./20 in M/10-HCl + 0·0509M-CH_2O	VI	(*a*)	1	2	5	7	9	
		(*b*)	1	4	8	12	17	100

(*b*) Methylenedinitroamine was over-neutralised with standard sodium hydroxide to give the disodium salt (M./20) in an excess (*ca.* 0·0012—0·0013M.) of alkali. On keeping the mixture at 25°, the alkalinity increased very slowly; after 459 hours, the mixture of run VII was 0·0032M., and that of run VIII (in which free formaldehyde was added initially) 0·0045M. with respect to free sodium hydroxide. In run VII, free formaldehyde, equivalent to the decomposition of 1% of the methylenedinitroamine, had appeared after 72 hours, but the amount did not increase thereafter; in run VIII the original concentration of formaldehyde remained unchanged. Since these results probably included the effect of the Cannizzaro reaction, the residual methylenedinitroamine was determined (Table V) by the

difference between the total formaldehyde present after complete decomposition (heating for 25 to 30 minutes at 80° in a buffer of pH 5·2) and the free formaldehyde present in a separate aliquot.

TABLE V.

Concentration of disodium methylenedinitroamine; solvent.	Run no.	% Decomposition. Hours at 25°.			
		1	72	240	459
M./20 in 0·0012M-NaOH	VII	0	1	1	4
M./20 in 0·0013M-NaOH + 0·0532M-CH_2O	VIII	1	2	4	6

G. Decomposition in Acetate Buffers.—Solutions of methylenedinitroamine in acetate buffers (2·5N-acetic acid–sodium hydroxide, with two exceptions) were maintained at 25°, and the amount of decomposition was determined by method (*b*) (section *D*). The results of these runs (IX to XXIX) are given in Figs. 1 and 2. Curve II of Fig. 1 shows clearly the decrease in the rate of decomposition with increased pH, but is not strictly comparable on account of the lowered buffering capacity. Some results in a phosphate buffer (2M-H_3PO_4—2N-NaOH) are recorded by curve V of Fig. 2.

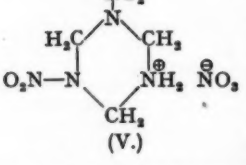
H. Further Investigation of the Decomposition in Unbuffered Solutions.—Solutions of methylenedinitroamine in water or dilute formaldehyde were maintained at 25°, and the decomposition was determined by methods (*a*) and (*b*) (section *D*). The results (of runs XXXII to XXXIX) represent an extension of section *D* to yield decomposition curves, which are given in Figs. 3 and 4. The difference between the upper and the lower line in each pair of curves provides an approximate measure of the nitroamine content of the solutions.

Preparation of 3 : 7-*Dinitro*-1 : 3 : 5 : 7-*tetra-aza*bicyclo[3 : 3 : 1]*nonane* (I) *from Methylenedinitroamine.*—Methylenedinitroamine (0·34 g. in 4 ml. of water) was partly neutralised with 2N-ammonia solution (1·2 ml.), and 38% formaldehyde (0·6 ml.) was added. After 24 hours, the precipitate (0·18 g.), m. p. 202° (decomp.), was collected and crystallised from nitromethane. 3 : 7-Dinitro-1 : 3 : 5 : 7-tetra-aza*bicyclo*[3 : 3 : 1]nonane (0·07 g.), m. p. 208—209° (decomp.), undepressed by admixture with an authentic specimen (Richmond, Myers, and Wright, *J. Amer. Chem. Soc.*, in press), was obtained. If no formaldehyde was added a small yield (0·03 g.) of material, m. p. 210°, was precipitated.

Determination of Free Methylenedinitroamine in the Presence of Acid and Formaldehyde.—To 10 ml. of solution, containing 0·1—0·4 g. of methylenedinitroamine, were added, successively, 9N-ammonia solution (5—6 ml.; note 1), M-barium chloride (5 ml.), and 90% methyl alcohol (30 ml.; note 2). After cooling for 1 hour in ice, the precipitate was collected on a sintered-glass funnel (the last traces were transferred with two 5-ml. portions of cold 60% methyl alcohol) and washed with, successively, cold 60% methyl alcohol (5 ml.), 90% methyl alcohol (5 ml.), and ether (15 ml.). Without being dried, the precipitate was then decomposed by stirring on the filter with an acetate buffer of pH 5·0 (25 ml.; 5—3 volumes of 2·5N-acetic acid and sodium hydroxide) and sucked through into a 100-ml. flask having a ground-glass neck. The solution and washings (25 + 15 ml. of water) were heated under reflux for 20 minutes at 80°, cooled, and made up to 250 ml. Formaldehyde (1 mol., equivalent to 1 mol. of methylenedinitroamine) was then determined iodometrically (note 3) in suitable (50 or 100 ml.) aliquots. An empirical correction (the addition of 11 mg. to the apparent weight of methylenedinitroamine in the original 10 ml. of solution) was required for accurate results.

Notes. (1) Added rapidly: pH at precipitation was >10. (2) All the methyl alcohol was purified by hypoiodite (Bates, Mullaly, and Hartley, *J.*, 1923, 403). A small quantity of ethyl alcohol (indicator solvent) in the original solution was not detrimental. (3) Blank determinations on the acetate buffer are recommended. 10—15 Minutes were ample for complete reaction when one-third of the iodine was available for back-titration, and an excess (*ca.* 1/20th of the total volume) of 2·5N-sodium hydroxide was added after decolorisation. Although ammonia interferes with the determination, any earlier coprecipitation of ammonium salts may be detected by the formation of nitrogen tri-iodide on the addition of sodium hydroxide.

In the following record of results, the empirical addition of 11 mg. is included and the true weight of methylenedinitroamine is given in parentheses. Mg. in 10 ml.: 402 (400); 199 (200); 115, 112 (114); 100 (100); 50, 54 (50). The following additional results were obtained after precipitation with 20 ml. of methyl alcohol, and washing with 50% methyl alcohol, the cooling time being 1—1½ hours. Mg. in 10 ml.: 403 (400); 199 (200); 100 (100); 47 (50).



(V.)

Determination of Potential Methylenedinitroamine.—(i) A suitable quantity of material was weighed into a small tared sintered-glass crucible, and decomposed by the addition of 6N-ammonia solution (7 ml.). After 5 minutes, with occasional stirring, the resultant liquid was sucked into a conical flask, and the crucible washed twice with 0·5N-ammonia solution (4 + 4 ml.). The insoluble material was weighed after vacuum-desiccation, and the methylenedinitroamine determined in the filtrate by addition of barium chloride etc., as previously detailed. (ii) Alternatively, if the material is wholly soluble, it may be decomposed in the precipitation flask by the addition of 3N-ammonia solution (15 ml.).

Samples (0·6—0·75 g.) of a specimen of 1 : 3-dinitrohexahydro-1 : 3 : 5-triazine nitrate (V) (Vroom and Winkler, *Canadian J. Res.*, in the press; also Bristol workers, private communication) were used. Method (i): Found: insoluble material, 0·5, 0·3%; potential methylenedinitroamine in soluble portion, 55, 56%. Method (ii): Found: potential methylenedinitroamine in total weight, 56, 55%. Calc. for (V): insoluble material, 0; potential methylenedinitroamine, 56·7%.

The authors' thanks are due to Mr. B. Skinner for a number of pH determinations.

THE UNIVERSITY, SHEFFIELD, 10. [*Received, December 15th*, 1948.]

358. *Studies on Nitroamines. Part VIII. The Dissociation Constants of the Primary Dibasic Nitroamines.*

By C. LINDLEY and J. C. SPEAKMAN.

Thermodynamic dissociation constants have been measured for the series of primary dibasic nitroamines, $NO_2{\cdot}NH{\cdot}[CH_2]_n{\cdot}NH{\cdot}NO_2$, in which $n = 1$, 2, 3, and 4. The resulting values of pK_1, pK_2, and ΔpK have been compared with those for corresponding members of the series of dicarboxylic acids, $CO_2H{\cdot}[CH_2]_n{\cdot}CO_2H$, on the basis of which comparison suggestions are made regarding the structure of the nitroamine anion.

THE ratio of the first to the second dissociation constant (K_1/K_2) of an acid with two equivalent acid groups in the molecule will be equal to 4, provided that these groups are so far apart as to exert no appreciable electrostatic influence upon one another. In other terms, ΔpK ($= pK_2 - pK_1$) = 0·60, where $pK = -\log K$. When, as is normally the case, the mutual electrostatic effect is not negligible, $\Delta pK > 0{\cdot}60$ and increases as the distance (r) between the charges on the acidic groups diminishes. Bjerrum (*Z. physikal. Chem.*, 1923, **106**, 219) attempted to put this relation into a quantitative form and deduced the equation, $\Delta pK - 0{\cdot}60 = e^2/2{\cdot}3kTDr$ where e is the electronic charge, k the Boltzmann constant, T the temperature, and D the dielectric constant of the solvent. This equation is only very approximately in agreement with observation, and attempts at a more sophisticated treatment (*e.g.*, by Ingold, *J.*, 1931, 2179; Kirkwood and Westheimer, *J. Chem. Physics*, 1938, **6**, 506; Wynne-Jones and Rushbrooke, *Trans. Faraday Soc.*, 1944, **40**, 99; see also *J.*, 1943, 270) have so far had only limited success. Nevertheless, it seemed possible that by comparing ΔpK values for two parallel series of dibasic acids, the structure in one of these series being known, useful inferences might be drawn regarding the separation of the negative charges on the anions of the other series. This empirical procedure would avoid many of the difficulties involved in deducing r directly from ΔpK.

Thermodynamic dissociation constants of the dinitroamines $NO_2{\cdot}NH{\cdot}[CH_2]_n{\cdot}NH{\cdot}NO_2$, where $n = 1$, 2, 3, and 4, have therefore been measured, and the ΔpK values have been compared with those for the corresponding dicarboxylic acids.

EXPERIMENTAL.

Thermodynamic dissociation constants were found by potentiometrically titrating the dinitroamines, in *ca.* 0·003M-aqueous solution, with 0·06N-NaOH at 20·0°, using the glass electrode. The methods of standardising the pH scale and of calculating K_1 and K_2 have been described previously (*J.*, 1940, 855). Results (expressed as pK) are shown in Table I; each value is the mean of at least five independent determinations. In general, they are considered to be accurate to within $\pm 0{\cdot}05$ for pK and rather better for ΔpK. Because of slow decomposition (see Part VII), the results for methylenedinitroamine may be a little less accurate than for the others.

Data for the dicarboxylic acids are also included; they are taken from the above-mentioned paper or from that of Gane and Ingold (*J.*, 1931, 2158).

TABLE I.

Thermodynamic dissociation constants at 20·0°.

Dinitroamine.	pK_1.	pK_2.	ΔpK.	Acid.	pK_1.	pK_2.	ΔpK.
Methylene	5·0	6·6	1·6	Malonic	2·83	5·69	2·86
Ethylene	5·35	6·66	1·31	Succinic	4·22	5·67	1·45
Trimethylene	5·59	6·85	1·26	Glutaric	4·39	5·50	1·11
Tetramethylene	5·87	6·98	1·11	Adipic	4·43	5·42	0·99

DISCUSSION.

The results in Table I show clearly that ΔpK diminishes much less rapidly as the homologous series of dinitroamines is ascended than it does in the dicarboxylic acid series. Qualitatively, therefore, the effective distance between the charges on the double anion increases more slowly in the former series.

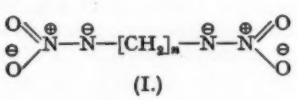
This conclusion can be extended semi-quantitatively by the following procedure. It seems probable that " straight-chain " molecules of the type considered here are most stable when in the fully extended form with the carbon chain in a flat zig-zag (Gane and Ingold, *J.*, 1931, 2158; Pauling, " The Nature of the Chemical Bond," 1942, p. 91; see also *J.*, 1941, 492). If so, the distance (r) between the negative charges on the double anions of the carboxylic acids can be easily calculated (Gane and Ingold, *loc. cit.*). If the values of ΔpK and r (columns 2 and 3 of Table II) are plotted graphically, the relation between them is thereby established on a purely

empirical basis. [Consideration of the Bjerrum equation suggests that it might be more convenient to plot ($\Delta pK - 0{\cdot}60$) against $1/r$; and in fact both methods of plotting have been used.] Now it appears reasonable to assume that the same, or a very similar, relation should apply in the series of dinitroamines; and with the use of this assumption empirical values of r_e, the effective separation of the charges, have been deduced and are shown in column 7 of Table II. Distances are given in A.

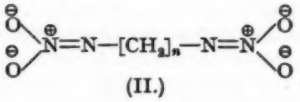
TABLE II.

Acid.	ΔpK.	r.	Dinitroamine.	ΔpK.	r_I.	r_e.	r_{II}.
Malonic	2·86	4·26	Methylene	1·6	2·4	5·6	6·2
Succinic	1·45	5·87	Ethylene	1·31	3·8	6·1	7·7
Glutaric	1·11	6·84	Trimethylene	1·26	5·0	6·3	8·4
Adipic	0·99	8·36	Tetramethylene	1·11	6·3	6·8	10·2

A possible interpretation of these values is provided by considering the formulation of the dinitroamine ion, viz. :



(I.)

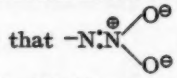
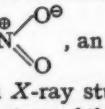


(II.)

Presumably resonance occurs between such forms as (I) and (II). If methylenedinitroamine existed mainly in form (I), the negative charges on the two inner nitrogen atoms would be separated by only about 2·5 A., giving rise to a powerful mutual repulsion. Consequently, this anion should exist mainly in form (II), where r is effectively much greater. As the homologous series is ascended, this repulsion would become less pronounced, with the result that the resonance would involve an increasing contribution from form (I).

This conclusion may be amplified by calculating values of r_I (minimum) and r_{II} (maximum), corresponding to formulæ (I) and (II), as shown in Table II (accepted values of interatomic distances and valency angles have been used). It is not at once obvious just where, on each $-N_2\overset{\ominus}{O}_2$ group, is located the effective negative charge which influences the loss or recapture of a proton by the other group; but, in a very approximate treatment, it seems justifiable to take this charge as being on the inner nitrogen atom in (I) and at the centre of the O–N–O triangle in (II). Comparison with the values of r_e supports the conclusion that the methylenedinitroamine and tetramethylenedinitroamine anions exist mainly as (II) and (I), respectively.

In general it is concluded that the normal condition of the nitroamine group is $-NH{\cdot}NO_2$, giving the anion $-\overset{\ominus}{N}{\cdot}NO_2$, but that, when two such groups are close enough together for the repulsion between their negative charges to become important, the anion exists mainly as $-N{:}\overset{\ominus}{N}O_2$, formally derived from the group $-N{:}NO{\cdot}OH$.

In the series of dicarboxylic acids, pK_1 increases with n, but pK_2 diminishes, whilst with the dinitroamines both pK_1 and pK_2 increase, although the latter increases more slowly. This is consistent with the above conclusion if it is assumed that  accepts a proton less readily than does  , an assumption that seems justifiable on general grounds.

On the basis of an *X*-ray study of the crystals, Llewellyn and Whitmore (*J.*, 1948, 1316) have discussed the structure of the ethylenedinitroamine *molecule*; and they tentatively conclude that it is a resonance hybrid involving important contribution from structures with double positive formal charges on the nitrogen atoms. It appears unlikely that the corresponding structures can make any significant contributions in the *anion*.

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359. *Quantitative Analysis of Mixture of Sugars by the Method of Partition Chromatography. Part III. Determination of the Sugars by Oxidation with Sodium Periodate.*

By E. L. Hirst and J. K. N. Jones.

Quantitative analysis, on the micro-scale, of reducing hexoses, pentoses, and methyl pentoses can be carried out by determining the formic acid produced when the sugars are oxidised with warm sodium periodate solution.

Determination, on the micro- or semi-micro-scale, of sugars separated on sheet filter paper by partition chromatography can be carried out by oxidation with Somogyi's copper reagent (*J. Biol. Chem.*, 1945, **160**, 61) or sodium hypoiodite, and the latter method can be used also for the micro-determination of the methylated aldose sugars (Jeanloz, *Helv. Chim. Acta*, 1946, **29**, 57; Hough, Hirst, and Jones, this vol., p. 928). Nevertheless, the use of these reagents suffers from disadvantages. For example, the copper reagent must be standardised against each individual sugar, whilst the alkaline hypoiodite reagent is not applicable to ketoses and does not oxidise quantitatively rhamnose and mannose. Sodium periodate oxidises compounds containing hydroxyl groups on adjacent carbon atoms with the formation of aldehydes and sodium iodate. If the resulting aldehydes possess a hydroxyl group on the carbon atom adjacent to the aldehyde group, further oxidation ensues with the formation of formic acid. For example, glycerol is converted quantitatively into two molecules of formaldehyde and one of formic acid on oxidation with periodates. The reducing hexose, pentose, and methyl pentose sugars contain hydroxyl groups on contiguous carbon atoms and would be expected to give formic acid on oxidation with sodium periodate, hexoses yielding five molecules, pentoses and methyl pentoses four molecules, and keto-hexoses three molecules of formic acid. Determination of the liberated formic acid is a relatively simple procedure, since it has been shown (Dunstan, Brown, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785; Halsall, Hirst, and Jones, *J.*, 1947, 1399) that, after addition of excess of ethylene glycol, the formic acid can be titrated in the normal manner, using methyl-red or screened methyl-red as indicator. Since oxidation of an aldose with alkaline hypoiodite leads to the consumption of only two equivalents of iodine, oxidation with sodium periodate should, other things being equal, give more accurate results and enable smaller quantities of sugars to be determined. The procedure outlined below is also quicker and simpler. It has been shown (Bell, *J.*, 1948, 992; Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941), that at ordinary temperatures the oxidation of the sugars and their methyl derivatives requires, in some cases, many hours for completion. To overcome this difficulty, oxidations were carried out on the boiling water-bath for 20 minutes, using a slight excess of 0·25M-sodium periodate. If too great an excess of the reagent is used, undue loss of formic acid may occur, and on the other hand, if too little is used, the reaction is not completed in 20 minutes. As a result of many experiments, it was found that 1 c.c. of 0·25M-sodium periodate was sufficient to oxidise, in 20 minutes, amounts of sugar ranging from 0·08 to 3·9 mg. in 6 c.c. of solution (see Tables I and III for results). Subsequent oxidation of the formic acid and of the formaldehyde produced in the reaction occurs to a small extent under the conditions employed but, even so, the yield of formic acid is about 96%. Sorbose gives low yields of acid and requires oxidation for a longer time before a 96% yield of formic acid results. Under the conditions used, the amount of formic acid produced from the liberated formaldehyde is so small that it may be neglected.

The oxidation of several derivatives of the simple sugars was also examined. Galacturonic acid yielded 96% of the expected amount of formic acid (five molecules) in 20 minutes, and the carbon dioxide produced in the reaction did not interfere with the determination. Glucosamine hydrochloride on oxidation should yield five molecules of formic acid, one of formaldehyde, and one of ammonium chloride, which could react with the formaldehyde yielding hexamine and might therefore be expected to lead to ambiguous results; in fact, however, 99% of the theoretical yield of acid was produced. Inositol behaved abnormally, yielding a low but consistent value of 75% of the expected quantity of acid (6 molecules); anomalous results with inositol have been encountered by other workers (Fleury, Poirot, and Fievet, *Compt. rend.*, 1945, **220**, 664).

Oxidation of partly methylated sugar derivatives gave ambiguous results, in some case more, and in others less, than the theoretical quantities of acid being produced. For example, 3 : 4-dimethyl rhamnose, which was expected to yield one molecule of acid, gave a 35% yield,

whilst 2 : 3-dimethyl glucose gave two molecules of formic acid when only one was to be expected (cf. Bell, *J.*, 1948, 992) The method is thus not satisfactory for the determination of methylated sugars.

As an example of the application of the method, a mixture of rhamnose, ribose, arabinose, and galactose was separated on the paper chromatogram and the sugars were determined by the method described above. Recoveries of 106—102% of the amounts actually present in the original mixture were achieved, when ribose is used as the reference sugar.

TABLE I.

2 C.c. of 0·25M-sodium periodate. Total vol., 7 c.c.

Sugar.	Duration of oxidation (mins.).	Amount of sugar taken (mg.).	Yield of formic acid.		
			mg.	mols./mol.	%.
Ribose	20	0·795	0·915	3·76	94
	40	0·795	0·935	3·84	96
	60	0·795	0·895	3·68	92
Arabinose	15	0·548	0·633	3·80	95
	15	0·822	0·950	3·80	95
	15	1·370	1·562	3·76	94
	30	0·274	0·312	3·76	94
	30	1·370	1·580	3·80	95
	20	0·086	0·0915	3·48	87
	20	0·259	0·296	3·72	93
	20	0·431	0·517	3·92	98
	40	0·086	0·096	3·64	91
	40	0·259	0·302	3·80	95
	40	0·431	0·491	3·72	93
Xylose	5	0·589	0·476	2·64	66
	20, 40, & 60	0·589	0·722	4·00	100
Rhamnose hydrate	10	0·738	0·708	3·80	95
	20	0·738	0·737	3·96	99
	30	0·738	0·715	3·84	96
	40	0·738	0·693	3·72	93
	20	1·320	1·268	3·84	96
Glucose	10	0·715	0·836	4·60	92
	20	0·715	0·831	4·60	92
	40	0·715	0·869	4·80	96
Galactose	10	0·725	0·792	4·30	86
	20	0·725	0·844	4·55	91
	40	0·725	0·895	4·80	96
	60	0·725	0·860	4·65	93
Mannose	20 & 40	1·00	1·214	4·80	96
Fructose	20, 40, & 60	0·895	0·653	2·85	95
Sorbose	10	0·463	0·315	2·64	88
	20	0·463	0·346	2·91	97
	40	0·463	0·346	2·91	97
	60	0·463	0·323	2·70	90
Galacturonic acid	20	0·741	0·846	4·80	96
	40	0·741	0·731	4·40	88
	60	0·741	0·621	3·50	70
Glucosamine hydrochloride	20	1·10	1·158	4·95	99
	40	1·10	1·038	4·45	89
	60	1·10	0·975	4·25	85
Sucrose	29, 40, & 60	0·487	0·0645	0·99	99
Inositol	20	0·416	0·488	4·50	75
	40	0·416	0·459	4·32	72
	60	0·416	0·484	4·56	76

TABLE II.

Oxidation of methylated sugars.

2 C.c. of 0·25M-sodium periodate. Total vol., 7 c.c.

Sugar.	Duration of oxidation (mins.).	Amount of sugar taken (mg.).	Yield of formic acid.	
			mg.	mols./mol.
6-Methyl glucose	20	0·99	0·861	3·60
	40	0·99	0·833	3·52
	60	0·99	0·766	3·24
3-Methyl glucose	20	0·672	0·242	1·52
	40	0·672	0·318	2·00
	60	0·672	0·318	2·00
2 : 6-Dimethyl galactose	20	0·662	0·233	1·52
	40 & 60	0·662	0·287	1·96
2 : 3-Dimethyl glucose	20	1·172	0·560	2·16
	40	1·172	0·545	2·10
	60	0·586	0·267	2·06
4 : 6-Dimethyl glucose	20	1·018	0·504, 0·517	2·24, 2·30
	40	1·018	0·575, 0·575	2·56, 2·56
	60	1·018	0·585, 0·607	2·60, 2·70
3 : 4-Dimethyl rhamnose	20	0·802	0·067	0·35
	40	0·802	0·100	0·52
	60	0·802	0·117	0·61

TABLE III.

1 C.c. of 0·25M-sodium periodate. Duration of oxidation, 20 mins. Total vol., 6 c.c.

Sugar.		Yield of formic acid.		
	mg.	mg.	mols./mol.	%.
Ribose	0·274	0·321	3·84	96
	1·369	1·545	3·68	92
Arabinose	0·086	0·157	4·04	101
	0·431	0·512	3·92	98
Xylose	0·201	0·252	4·08	102
	1·205	1·410	3·84	96
Rhamnose hydrate	0·156	0·157	3·96	99
	0·782	0·786	3·96	99
	3·91	3·93	3·96	99
Glucose	0·251	0·317	4·95	99
	1·254	1·535	4·80	96
Mannose	0·211	0·262	4·85	97
	1·06	1·355	4·75	95
Galactose	0·244	0·258, 0·266 *	4·50, 4·65 *	90, 93 *
	1·12	1·33, 1·336 *	4·65, 4·70 *	93, 94 *
Fructose	0·225	0·162	2·82	94
	1·123	0·780	2·70	90
Sorbose	0·195	0·146, 0·156 *	2·94, 3·00 *	98, 100 *
	0·976	0·594, 0·588 *	2·40, 2·37 *	80, 79 *
Dulcitol	0·211	0·210	3·96	99
	1·053	1·061	3·96	99

* After 40 minutes' oxidation.

EXPERIMENTAL.

General Procedure.—Reactions were carried out in test-tubes, 22 × 3 cm., fitted with a B-34 ground joint and stopper. The apparatus was cleaned with chromic acid and well washed with distilled water before use.

Sodium metaperiodate was prepared from sodium paraperiodate by recrystallisation from nitric acid. It contained no free acid since, on addition of excess of ethylene glycol to its aqueous solution, sodium iodate neutral to methyl-red was formed. The ethylene glycol was neutral to methyl-red.

The sugar (0·2—3 mg.) was dissolved in water (*ca.* 5 c.c.) and oxidised with *ca.* 0·25M-sodium metaperiodate on the boiling water-bath for the requisite length of time. The solution was then cooled under the tap, and ethylene glycol (*ca.* 0·2 c.c.) added to destroy excess of periodate. The formic acid was titrated with 0·01N-sodium hydroxide, using methyl-red or screened methyl-red as indicator. At the end-point of the titration, the colour of the solution was identical with that of a control solution containing ethylene glycol and indicator. Under these conditions formic acid is slowly destroyed, and formaldehyde is slowly oxidised to formic acid, but neither reaction is sufficiently rapid to invalidate the method (see below).

Control Experiments with Formaldehyde.—An aqueous solution of ethylene glycol (0·005M.; 5 parts) was oxidised with sodium metaperiodate solution (2 parts) on the boiling water-bath. This gave a solution 0·01M. with respect to formaldehyde. At intervals, samples were cooled under the tap, excess of glycol was added, and the formic acid was titrated. Found, c.c. of 0·01N-acid per 5 c.c. of ethylene glycol solution: 0·15 (20 minutes), 0·38 (40 minutes), 0·63 (1 hour).

Control Experiments with Formic Acid.—Portions of 0·01N-formic acid (5 c.c.) were oxidised with sodium metaperiodate (2 c.c.) on the boiling water-bath for the requisite length of time. The sample was cooled, the periodate was destroyed, and the formic acid was titrated. Found: c.c. of 0·01N-formic acid: 4·9 (20 minutes), 4·7 (40 minutes), 4·7 (1 hour).

In a second experiment, samples containing 0·01N-formic acid (1 c.c.), water (4 c.c.), and sodium metaperiodate (2 c.c.) were heated on the boiling water bath. Found: c.c. of 0·01N-formic acid: 0·94 (20 minutes), 0·91 (40 minutes), 0·84 (1 hour).

The experiments were repeated using less sodium periodate solution (1 c.c.) in order to lower the rates of oxidation of formaldehyde and formic acid.

Control Experiments with Formaldehyde.—Solutions of ethylene gycol (0·005M) were oxidised with sodium metaperiodate (1 c.c.), yielding solutions of formaldehyde (0·01N.) which were then heated for the requisite time. Found, c.c. 0·01N-acid produced: (*a*) 0·01 c.c. from 1 c.c. of solution, diluted with water (4 c.c.) after 20 minutes and 40 minutes. (*b*) 0·10 c.c. from 5 c.c. of solution after 20 minutes and 40 minutes.

Control Experiments with Formic Acid.—Formic acid (5 c.c., 0·01N.) was heated with sodium metaperiodate (1 c.c.) for the requisite time. Found, % recovery of formic acid: 1 c.c. of formic acid solution gave 95% and 94% yield after 20 and 40 minutes, respectively; 5 c.c. of formic acid solution gave 96% and 92% yield after 20 and 40 minutes, respectively. These experiments show that the recovery of formic acid is not dependent on its concentration.

Separation and Determination of a Mixture of Ribose, Arabinose, Galactose, and Rhamnose.—A mixture of ribose (18·47 mg.), arabinose (20·48 mg.), galactose (24·02 mg.), and rhamnose (36·50 mg.) was dissolved in water (2 c.c.), and the sugars were separated on the paper chromatogram by the method of Flood, Hirst, and Jones (*J.*, 1948, 1681). The sugars were extracted from the paper strips by placing the paper in the cup of a small Soxblet apparatus, fitted with B-34 ground joints and of the type described by A. A. Morton ("Laboratory Technique," p. 202, McGraw-Hill Book Co. Inc., 1938). The blank due to the paper was never more than 0·10 c.c. of 0·01N-sodium hydroxide (Found: 0·07, 0·07, 0·05, 0·10, 0·06, 0·07). The sugars, thus obtained as solutions in water (*ca.* 5 c.c.), were oxidised with sodium metaperiodate solution (1 c.c.) on the boiling water-bath for 20 minutes. The solutions were then cooled, and the excess of sodium periodate was destroyed by the addition of ethylene glycol. Methyl-red (2 drops) was added and the formic acid titrated with 0·01N-sodium hydroxide. Found: ribose, 0·176 mg.; arabinose, 0·206 mg.; galactose, 0·241 mg.; rhamnose 0·355 mg. The amounts of arabinose, galactose, and rhamnose calculated to have been present in the original mixture are, therefore, 21·6 mg. (106% recovery), 25·3 mg. (104% recovery), and 37·2 mg. (102% recovery), respectively, ribose being used as the reference sugar.

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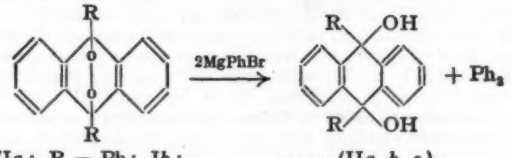
NOTES.

Reactions of Grignard Solutions. Part II. Action of the Grignard Reagent on Photo-peroxides of 9 : 10-*Diarylanthracene.* By AHMED MUSTAFA.

DUFRAISSE (*Bull. Soc. chim.*, 1933, [iv], **53**, 789) has obtained 5 : 12-dihydroxy-5 : 6 : 11 : 12-tetraphenyl-5 : 12-dihydronaphthacene by the action of Grignard reagents on 5 : 6 : 11 : 12-tetraphenylnaphthacene dioxide, an *isomerisation product* obtained by the action of magnesium iodide on an ethereal solution of the photo-peroxide of 5 : 6 : 11 : 12-tetraphenylnaphthacene (cf. Dufraisse, *loc. cit.*; Bergmann and Maclean, *Chem. Reviews*, 1941, **28**, 387). All attempts to rearrange the photo-peroxides of 9 : 10-diphenylanthracene (I*a*) in an analogous manner have failed (Dufraisse and Le Bras, *Bull. Soc. chim.*, 1937, [v], **4**, 349).

The photo-peroxide (I*a*) of 9 : 10-diphenylanthracene with phenylmagnesium bromide, in ether–benzene gives, after decomposition with ammonium chloride, 9 : 10-dihydroxy-9 : 10-diphenyl-9 : 10-dihydroanthracene (II*a*) in almost quantitative yield together with diphenyl, whereas (I*a*) is recovered unchanged when shaken with an aqueous solution of ammonium chloride for three hours at room temperature (cf. the action of water in dioxan in the presence of sulphuric acid; Pinnazi, *Compt. rend.*, 1947, **225**, 1012). 9 : 10-Dihydroxy-9 : 10-di-*p*-tolyl- (II*b*) and 9 : 10-dihydroxy-9 : 10-di-*m*-tolyl-

dihydroanthracene (II*c*) were obtained similarly from the photo-peroxides of 9 : 10-di-*p*-tolyl- (I*b*) and 9 : 10-di-*m*-tolyl-anthracene (I*c*), respectively.



(I*a*: R = Ph; I*b*: R = *p*-C_6H_4Me; I*c*: R = *m*-C_6H_4Me). (II*a*, *b*, *c*.)

This is an example of the breaking of the linkage by the action of Grignard reagent, resulting in reduction (cf. Crawford, *J. Amer. Chem. Soc.*, 1935, **57**, 2000; Kharasch and Weinhouse, *J. Org. Chem.*, 1936, **1**, 209).

Action of Phenylmagnesium Bromide.—(a) *On* 9 : 10-*diphenylanthracene* (*photo-*)*peroxide.* An ethereal solution of phenylmagnesium bromide [prepared from magnesium (1·8 g.), bromobenzene (18·8 g.), and dry ether (100 c.c.)] was filtered and divided into two portions. To one portion, (I*a*) (1·5 g.) and dry benzene (30 c.c.) were added. To the second portion, only dry benzene (30 c.c.) was added. The two portions were then boiled under reflux for two hours and kept overnight at room temperature, and then decomposed with cold aqueous ammonium chloride solution and extracted with ether. The ethereal solutions were washed with water, dried (Na_2SO_4), and evaporated. The oily residue obtained from the first portion was extracted several times with light petroleum (b. p. 30—50°); the insoluble solid material obtained after thorough washing with light petroleum crystallised from benzene in colourless crystals, m. p. 255—256° (1·2 g.; *ca.* 85%); it was 9 : 10-dihydroxy-9 : 10-diphenyldihydroanthracene (II*a*) (m. p., mixed m. p., and blue colour with concentrated sulphuric acid) (Found: C, 85·6; H, 5·4. Calc. for $C_{26}H_{20}O_2$: C, 85·7; H, 5·4%). The light-petroleum extract gave, on slow evaporation, diphenyl as colourless crystals, m. p. 71° (0·74 g.).

The oily residue obtained from the evaporation of the second ethereal solution solidified when cooled and scratched and was then recrystallised from light petroleum (b. p. 50—60°), giving diphenyl as colourless crystals, m. p. 71° (0·23 g.).

(b) *On* 9 : 10-*di*-p-*tolylanthracene* (*photo-*)*peroxide* (I*b*). (I*b*) (Willemart, *Bull. Soc. chim.*, 1 937, [v], **4**, 510) (1·5 g.), when similarly treated, gave 9 : 10-dihydroxy-9 : 10-di-*p*-tolyl-9 : 10-dihydroanthracene (II*b*) as colourless crystals, m. p. 273° from benzene (m. p., mixed m. p., and colour reaction with sulphuric acid) (Found: C, 85·8, H, 6·0. Calc. for $C_{28}H_{24}O_2$: C, 85·7; H, 6·1%). Ingold and Marshall (*J.*, 1926, 3080) gave m. p. 275° for (II*b*).

(c) *On* 9 : 10-*di*-m-*tolylanthracene* (*photo-*)*peroxide* (I*c*). Similarly, (I*c*) (Willemart, *loc. cit.*) (1·5 g.) afforded 9 : 10-dihydroxy-9 : 10-di-*m*-tolyl-9 : 10-dihydroanthracene (II*c*) as colourless crystals (from toluene), m. p. 247° (m. p., mixed m. p., and colour reaction with sulphuric acid) (Found: C, 85·6; H, 6·2).—FACULTY OF SCIENCE, FOUAD I UNIVERSITY, ABBASSIA, CAIRO, EGYPT. [*Received, November* 17*th*, 1948.]

The Preparation of Diacridyl by treating 5-Chloroacridine with Chromous Sulphate. By RICHARD ROYER.

THE reduction of 5-chloroacridine by chromous sulphate has been investigated. The use of chromous chloride has been recommended for reducing iminochlorides to Schiff bases by von Braun and Rudolph (*Ber.*, 1934, **67**, 269, 1735), and, as 5-chloroacridine may be considered a vinylogous iminochloride, it was anticipated that this method of reduction would yield acridine. However, no appreciable quantity of acridine or acridan was formed, but diacridyl was produced in almost quantitative yield.

A 0·1N-chromous sulphate solution in 0·1N-sulphuric acid was prepared by the simplified method of Lingane and Pectok (*Analyt. Chem.*, 1948, **20**, 425). This solution (100 ml.) was added, in an atmosphere of hydrogen, to a solution of 5-chloroacridine (0·50 g.; 0·0023 mole) in glacial acetic acid (9 ml.) at 20°. The change in colour from blue to green was immediate. After 30 minutes, concentrated sulphuric acid (2 ml.) and potassium dichromate (0·85 g.) were added, and the mixture was boiled for 20 minutes. After the mixture had been cooled, the solid was collected, washed thoroughly with water, and decomposed by aqueous ammonia, giving diacridyl (0·40 g.; 96%) [Found (after crystallisation from pyridine): C, 87·3; H, 4·4. Calc. for $C_{26}H_{16}N_2$: C, 87·6; H, 4·5%—microanalysis by The Wellcome Chemical Works, Dartford]. The product did not melt below 360° and was insoluble in alcohol (imparting no fluorescence to the wash-liquor); when potassium iodide was added to its solution in 0·5N-hydrochloric acid, the characteristic red iodide of diacridyl separated.—WELLCOME RESEARCH INSTITUTION, LONDON, N.W.1. [*Received, December* 9*th*, 1948.]

Relative Rates of Hydrogenolysis. By J. G. M. BREMNER and R. K. F. KEEYS.

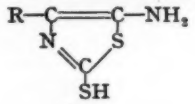
THE hydrogenolysis of furfuraldehyde to sylvan at atmospheric pressure in presence of a copper catalyst is inhibited by the presence of alkali, furfuryl alcohol being the main product formed (Bremner and Keeys, *J.*, 1947, 1068). The behaviour of acetophenone under similar conditions has now been examined. It has been found that a copper catalyst which leads to furfuryl alcohol in good yield does not show the same selectivity in the hydrogenation of acetophenone. The reaction does not stop when phenylmethylcarbinol is formed, but hydrogenolysis sets in with the formation of ethylbenzene and water. Thus 1·96 moles of hydrogen per mole of acetophenone were absorbed on passing the ketone at a

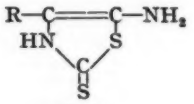
space velocity * of 0·32 hour^{-1}, with a 5·5-molar ratio of hydrogen, over the copper catalyst at 235°. Only a trace (1%) of unchanged ketone, estimated by oximation, was present in the product which was in two layers. Distillation gave a water–ethylbenzene fraction, followed by the main ethylbenzene fraction at 135°, whilst only 8% of higher-boiling material remained.

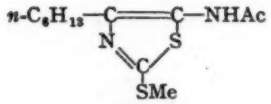
Since it has already been shown (*loc. cit.*) that those catalysts which reduce furfuraldehyde to sylvan do not convert acetone into propane, the following sequence (cf. Adkins, " Reactions of Hydrogen," Univ. of Wisconsin Press, 1937, p. 70) with regard to rate of reaction with hydrogen is observed: acetophenone > furfuraldehyde ⩾ acetone. If it be assumed that ions of the type, $^+$CHPhMe, $C_4H_3O{\cdot}CH_2^+$, and Pr^{i+}, are intermediates in these hydrogenolysis reactions (Bremner, *Research*, 1948, **1**, 281), their respective resonance energies will be in this, the expected, sequence.—IMPERIAL CHEMICAL INDUSTRIES LIMITED, BILLINGHAM DIVISION, BILLINGHAM, CO. DURHAM. [*Received, December 16th*, 1948.]

Thiol–thione Tautomerism of 5-Amino-2-mercaptothiazoles. By E. S. STERN.

DATA for the ultra-violet-light absorption of 5-amino-2-mercaptothiazoles (I, I*a*) in neutral and alkaline solution (see table) published recently (Cook *et al.*, *J.*, 1947, 1598; 1948, 201, 2031) permit the interpretation of an interesting case of functional-group isomerism influenced by the substituent in the 4-position of the thiazole nucleus.

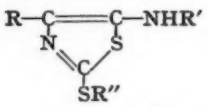

(I.)


(I*a*.)


(II.)

Of the two possible forms (I and I*a*), the thiol form (I) may be assumed to be favoured in alkaline solution. When R = H or an alkyl group (*e.g.*, *n*-C_6H_{13}, or CHEtBu^n), the ammoniacal solution of the thiazoles shows a light-absorption maximum at about 3040 A. This maximum ($\lambda_{max.}$ 2990 A.) is also observed for 5-acetamido-2-methylthio-4-*n*-hexylthiazole (II), a compound presumably existing entirely in the "thiol" form, in alcoholic solution. The absorption spectra of alcoholic solutions of 5-amino- and 5-acetamido-2-mercapto-4-alkylthiazoles, however, differ markedly from those of alkaline solutions and show maxima at about 3380 A., and at 3370 A., respectively. It may be concluded, therefore, that in alcoholic solution the thione form (I*a*) predominates.

Light Absorption Properties of Aminomercaptothiazoles.



				Neutral solution.		Alkaline solution.	
Ref.	R.	R′.	R″.	$\lambda_{max.}$	ε.	$\lambda_{max.}$	ε.
a	H	H	H	2280 A.	4,500	Unstable	
				3380	10,700		
b	*n*-C_6H_{13}	H	H	3340	11,200	3040 A.	5,500
b	CHEtBu^n	H	H	3390	11,500	3040	7,500
c	Ph	H	H	2230	12,500	2230	18,000
				2810		3060	12,000
				2900	8,500		
				3000			
b	*n*-C_6H_{13}	Ac	H	3370	16,000		
b	*n*-C_6H_{13}	Ac	Me	2500	3,600		
				2560	3,600		
				2990	12,000		

References: *a*, *J.*, 1948, 201; *b*, *J.*, 1948, 2031; *c*, *J.*, 1947, 1598.

5-Amino-2-mercapto-4-phenylthiazole (I; R = Ph), on the other hand, has its light-absorption maximum of longest λ in dioxan and in alkaline solution at 3000 A. and at 3060 A., respectively, and thus appears to exist in the thiol form (I) in neutral solution.

The fact that these differences in light absorption are accompanied by great differences in melting point and other physical properties, and by differences in reactivity towards methylating agents (*e.g.*, diazomethane) in alcoholic solution, lends support to the view that this is a case of tautomerism in which the substituent at the 4-position plays a decisive role in determining the final form assumed by the ring system, and which may be explained in the following terms. The thiazole ring system is now generally accepted as being aromatic in character (see Erlenmeyer *et al.*, *Helv. Chim. Acta*, 1948, **31**, 1978) and probably exists in the form of a resonance hybrid in which the classical single and double bonds interact to a large extent (cf. Metzger and Pullman, *Compt. rend.*, 1948, **226**, 1613). In the thiazoline structure (I*a*) there are two single bonds between $C_{(4)}$ and $C_{(5)}$, and this tautomer would not be expected to conjugate with a substituent at $C_{(4)}$; in the resonating thiazole structure (I), on the other hand, there should

* Space velocity refers to the volume of acetophenone fed per unit bulk volume of catalyst space per hour.

be no obstacle to conjugation with a substituent at $C_{(4)}$, and the structure (I) should be favoured by $C_{(4)}$-substituents with great conjugating power. The conjugating power of the phenyl substituent is evidently sufficient, and that of an alkyl substituent is insufficient, to force the hetero-ring into the thiazole form (I) capable of conjugation. The author thanks Sir Ian Heilbron and Dr. A. H. Cook for their kind interest and encouragement.—IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON, S.W.7. [*Received, December 21st,* 1948.]

Some Guanidine and Diguanide Derivatives. By RICHARD ROYER.

IN the course of studies on the relation between chemical constitution and antibacterial properties, the need arose for a series of aryl-guanidines and -diguanides having different areas of flat surface. This involved the preparation of the following compounds not previously described, *viz.*, 2-*anthryl-* and 3-*acridyl-guanidine* and N¹-2-*anthryl-*, N¹-6-*quinolyl-*, and N¹-3-*acridyl-diguanide*. 2-Aminoanthracene was treated with methyl*iso*thiourea sulphate, and 3-aminoacridine was condensed with cyanamide, to form the corresponding guanidines. The diguanides were prepared by treating the appropriate amine hydrochlorides with dicyandiamide.

2-*Anthrylguanidine*.—2-Aminoanthracene (1·0 g.), methyl*iso*thiourea sulphate (3·7 g.; 5 equivs.) and a trace of copper sulphate were heated in *p*-cresol (5 g.) at 200—205° for 45 minutes, whereafter evolution of methanethiol ceased. The cooled mass was extracted with ether to remove *p*-cresol, and the residue was basified with excess of 60% sodium hydroxide solution. The mixture of bases was made acid to litmus with N-acetic acid, boiled, and filtered, and the residue extracted with boiling water until no further solid was obtained on basifying the extract. The combined filtrates, on basification with 60% sodium hydroxide solution, gave 0·64 g. (51%). Crystallisation from alcohol (75 parts) yielded 2-*anthrylguanidine* as yellow crystals, m. p. 215° (decomp.) (Found: C, 76·0; H, 5·6; N, 17·8. $C_{15}H_{13}N_3$ requires C, 76·55; H, 5·6; N, 17·85%). Solutions of the base do not display the green fluorescence of 2-aminoanthracene; the mono-ion is colourless, the solution fluorescing violet. The hydrochloride is moderately soluble in cold water.

2-Naphthylguanidine, similarly prepared in 30% yield, had m. p. 143·5° (from benzene). Arndt and Rosenau (*Ber.*, 1917, **50**, 1261) gave m. p. 140°.

3-*Acridylguanidine*.—3-Aminoacridine dihydrochloride (2·7 g.) and cyanamide (3·7 g.) in absolute alcohol (15 ml.) were heated for 7 hours in a sealed tube in a bath at 130—135°. The dark mass was evaporated to dryness, the residue basified with 60% sodium hydroxide solution, and the resulting tar washed with water and dried (in a desiccator; KOH). The tar was extracted with N-acetic acid, and the extract made neutral to litmus with ammonia, liberating a red oil (*A*). The liquor was separated and made alkaline to Orange-II paper with 60% sodium hydroxide solution, precipitating a tar. The oil (*A*) was submitted to the above process repeatedly until final basification was not productive. The precipitated tars were collected and crystallised twice from alcohol, giving 0·5 g., m. p. 203° (decomp.) (15%). Recrystallisation from alcohol (15 parts) yielded 3-*acridylguanidine* as solvated yellow crystals, m. p. 206° (decomp.) (Found: C, 67·1; H, 6·8; N, 18·2. $C_{14}H_{12}N_4,1\frac{1}{2}C_2H_6O$ requires C, 66·8; H, 6·9; N, 18·4%). The base does not fluoresce in solution, and the mono-ion is yellow, displaying a faint violet fluorescence in dilute solution (3-aminoacridine shows an intense green fluorescence in solution and has a red mono-ion). The acetate and hydrochloride are freely soluble in water.

N¹-2-*Anthryldiguanide*.—2-Aminoanthracene hydrochloride (6·8 g.), dicyandiamide (2·5 g.; 1 equiv.), and alcohol (30 ml.) were heated for 7 hours in a sealed tube in a bath at 125—130°. The solid was washed with alcohol and shaken in a stoppered flask with excess of 60% sodium hydroxide solution; the resulting mixture of bases was dried and extracted with boiling toluene (100 ml.) to remove unchanged 2-aminoanthracene. The residue was extracted with boiling alcohol (3 l.), and the extract concentrated to crystallising-point, yielding the required base (4·2 g.; 51%). Recrystallisation from alcohol (400 parts) gave N¹-2-*anthryldiguanide* as pale yellow crystals, m. p. 214° (decomp.) (Found: C, 69·4; H, 5·5; N, 25·1. $C_{16}H_{15}N_5$ requires C, 69·25; H, 5·5; N, 25·25%). Solutions of the base do not fluoresce and the mono-ion is colourless. The hydrochloride is sparingly soluble in cold water. Attempts to effect the reaction in water, glycerol, or ethylene glycol were less successful.

N¹-6-*Quinolyldiguanide*.—6-Aminoquinoline (7·2 g.), dicyandiamide (4·2 g.; 1 equiv.), and 3N-hydrochloric acid (30 ml.) were heated under reflux for 2 hours; after concentration of the solution to one-third of its volume, the addition of alcohol (120 ml.) precipitated the hydrochloride (6·5 g.). To a solution of this salt (2·65 g.) in water (8 ml.) was added 60% sodium hydroxide solution (4·5 ml.), precipitating an oil which quickly solidified (1·83 g., 34%). Crystallisation from alcohol (3 parts) gave N¹-6-*quinolyldiguanide* as pale buff crystals, m. p. 188° (decomp.) (Found: C, 57·45; H, 5·3; N, 36·6. $C_{11}H_{12}N_6$ requires C, 57·9; H, 5·3; N, 36·8%). The base is soluble in *ca.* 20 parts of cold and 4 parts of boiling water, or in 10 parts of cold alcohol; it is sparingly soluble in ether or benzene. The solution in 3N-hydrochloric acid fluoresces bright yellow in ultra-violet light.

N¹-3-*Acridyldiguanide*.—3-Aminoacridine (2·2 g.), dicyandiamide (1·0 g.; 1 equiv.) and 2N-hydrochloric acid (8·8 ml.) were heated under reflux for 2 hours, and the solution cooled to 60°. Sodium hydrogen carbonate (3·3 g.) in water (10 ml.) then precipitated a tar which was extracted with water (at 60°) until it solidified, leaving unchanged 3-aminoacridine (0·95 g.). The combined filtrates were acidified and boiled to expel carbon dioxide, and the crude base was precipitated by the addition of excess 60% sodium hydroxide solution. A second sodium hydrogen carbonate treatment (as above) removed a further trace of 3-aminoacridine. The precipitated base was then crystallised from alcohol (60 parts), giving yellow crystals of N¹-3-*acridyldiguanide*, m. p. 202° (decomp.) (yield, 13%) (Found: C, 64·8; H, 5·1; N, 29·9. $C_{18}H_{14}N_6$ requires C, 64·7; H, 5·1; N, 30·2%). Solutions of the base do not fluoresce and the mono-ion is yellow. The yield was not improved by prolonging the reaction time or by using a two-fold excess of dicyandiamide.

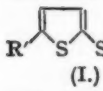
The dissociation constants of these bases were determined potentiometrically in 50% alcohol at 20° by Mr. J. N. Phillips, M.Sc., University of Sydney, by whose kind permission they are reproduced here,

viz.: 2-naphthyl- 10·7, 2-anthryl- 11·0, and 3-acridyl-guanidine 10·3; N^1-2-anthryl- 10·4, and N^1-6-quinolyl-diguanide 10·7. It was not possible to obtain an accurate figure for N^1-3-acridyldiguanide because of a strong tendency to form micelles, even in dilute solution.

The author thanks Dr. A. Albert for help and criticism and Miss J. Fildes, B.Sc., for microanalyses.—THE UNIVERSITY, SYDNEY. [*Received, January 8th,* 1949.]

Some 2-Thienyl Alkyl Sulphones. By J. CYMERMAN and J. L. LOWE.

THE antibacterial properties of many *p*-substituted phenyl alkyl sulphones are well known, and preliminary model experiments directed towards the synthesis of thiophen analogues, *e.g.*, [I; R = NH_2, $CH_2{\cdot}NH_2$, or $C(:NH){\cdot}NH_2$; R″ = alkyl or aralkyl] have now been carried out.



(I.)

Several aryl thienyl sulphones have been described (*e.g.*, Bambas, *J. Amer. Chem. Soc.*, 1945, **67**, 668; Burton and Davy, *J.*, 1948, 525), and Burton and Davy (*loc. cit.*) also prepared 2-thienyl methyl sulphone. This compound was made independently in this laboratory, along with a series of homologues (I; R′ = H; R″ = alkyl or aralkyl), which is listed in the table. The method used in each case was the alkylation of sodium thiophen-2-sulphinate. Attempts to prepare the hexadecyl compound gave an unidentified solid, m. p. 35°.

Decomposition of an aqueous solution of thiophen-2-sulphinic acid, catalysed by hydriodic acid, afforded *di(2-thienyl) disulphoxide*, m. p. 46—47°.

The molecular refractivities of the liquid sulphones were determined, using a micro-method, accurate to about 1%, to determine the densities. The figures (see table) are in fair agreement with those calculated from the atomic refractivities of sulphur, in its several valency states, given by Strecker and Spitaler (*Ber.*, 1926, **59**, 1754).

Alkyl Group.	Yield, %.	B. p./mm.	n_D^t.	d_4^t.	Formula.	$[R_L]_D$. Found.	$[R_L]_D$. Calc.	Analysis, %. Found. C.	Found. H.	Reqd. C.	Reqd. H.
Methyl	36	(m. p. 47°)	—	—	$C_5H_6O_2S_2$	—	—	37·5	3·4	37·05	3·7
Ethyl	27	120°/2·5	1·5507 (18°)	1·297 (18°)	$C_6H_8O_2S_2$	43·39	43·18	40·95	4·25	40·9	4·55
n-*Propyl*	70	153—154°/6·5	1·5404 (20°)	1·259 (20°)	$C_7H_{10}O_2S_2$	47·33	47·80	44·25	5·3	44·5	5·25
n-*Butyl*	29	163—165°/3	1·5369 (15°)	1·199 (15°)	$C_8H_{12}O_2S_2$	52·98	52·42	47·1	6·05	47·1	5·9
n-*Amyl*	33	162—163°/2	1·5282 (17°)	1·181 (17°)	$C_9H_{14}O_2S_2$	56·80	57·04	49·5	6·05	49·55	6·4
n-*Hexyl*	30	160°/2	1·5230 (15°)	1·176 (15°)	$C_{10}H_{16}O_2S_2$	61·20	61·66	51·7	7·0	51·7	6·9
4-*Nitrobenzyl*	9	(m. p. 177°)	—	—	$C_{11}H_9O_4NS_2$	—	—	N 5·3	—	N 4·95	—

Thiophen-2-sulphinic Acid (*cf.* Smiles and Bere, *J.*, 1924, 2361).—Thiophen-2-sulphonyl chloride (9·15 g.) (Steinkopf and Hopner, *Annalen*, 1933, **501**, 174) in acetone (10 c.c.) was slowly added to a vigorously stirred solution of sodium sulphite heptahydrate (25·2 g.) in water (120 c.c.) at 60°. The solution was maintained at pH 8—9 by suitable additions of solid sodium hydrogen carbonate, and stirred at 60° for 2 hours after the addition of sulphonyl chloride was complete. After cooling, the solution was acidified with 2N-sulphuric acid and extracted with ether. The ethereal extract was shaken with dilute sodium hydrogen carbonate solution (150 c.c.), and the aqueous solution of sodium thiophen-2-sulphinate thus obtained (0·041 mol., 81% of theory; amount estimated in an aliquot part by acidifying, extracting the sulphinic acid with chloroform, and titrating with standard sodium hydroxide solution) was used in subsequent preparations.

Preparation of Sulphones.—A solution (pH 8—9) of sodium thiophen-2-sulphinate (0·02 mol.) in water (70 c.c.) was heated under reflux for 12—18 hours with the alkyl halide (0·6 mol.) and sufficient alcohol to render the hot solution homogeneous. Excess of alkyl halide and alcohol were distilled off, and the sulphones isolated by ether extraction. The crude products were usually purified by vacuum-distillation, except the methyl and *p*-nitrobenzyl compounds, which were crystallised from aqueous alcohol.

Di-(2-thienyl) Disulphoxide.—A solution of sodium thiophen-2-sulphinate (0·01 mol.) in water (35 c.c.) was acidified with 2N-sulphuric acid, and 10% hydriodic acid (0·1 c.c.) was added. The mixture was set aside at room temperature for 3 days, whereupon white crystals gradually separated. Extraction with ether, and removal of solvent from the washed (sodium hydrogen carbonate) and dried extract, afforded a residue of di-(2-thienyl) disulphoxide, crystallising from aqueous alcohol in white needles, m. p. 46—47° (0·31 g.; 36%) (Found: C, 36·55; H, 2·2. $C_8H_6O_2S_4$ requires C, 36·65; H, 2·3%).

The authors thank Mr. J. R. Rowlands for his interest and encouragement and Dr. W. F. Short and Messrs. Boots Pure Drug Co. Ltd. for kindly affording facilities for analyses.—NOTTINGHAM AND DISTRICT TECHNICAL COLLEGE, NOTTINGHAM. [*Received, January 22nd,* 1949.]

OBITUARY NOTICES.

THOMAS STEWART PATTERSON.

1872—1949.

THOMAS STEWART PATTERSON was born at Greenock in July, 1872, and died on February 14th, 1949, at his home in Glasgow, close to the precincts of the University he had served with devotion and distinction for nearly forty years. The son of a chemist—and in course of time, writer of his father's Obituary Notice for the Journal (Thomas Law Patterson, *J.*, 1916, **109**, 387)—he was educated at Greenock Academy and at Merchiston Castle School, Edinburgh, before beginning the serious study of chemistry, first under W. Dittmar at the Andersonian College, Glasgow, and then under V. Meyer at Heidelberg. In 1895 he graduated Ph.D. at Heidelberg, with a Thesis entitled, "Ueber Iodoso- und Iodo-benzaldehyde." The ensuing year he spent as Priestley Scholar, under P. F. Frankland, at Mason College, Birmingham, and here was aroused an interest in stereochemistry, and particularly in optical activity, that lasted through his lifetime. From 1896 to 1904 he acted first as Demonstrator and later as Assistant Lecturer in the Yorkshire College, Leeds, and then transferred to Glasgow University where he combined the duties of Senior Assistant to the Regius Professor, John Ferguson, with a Lectureship in Organic Chemistry. During an inter-regnum following Ferguson's retirement in 1915, Patterson had general charge of the Chemistry Department until in 1919, with G. G. Henderson succeeding to the Regius Chair, he himself was appointed to the newly founded Gardiner Chair of Organic Chemistry, which he held until his retirement in 1942. He was D.Sc. of London (1909) and Glasgow (1911), and, shortly after his retirement, the title of Emeritus Professor and the LL.D. of Glasgow were conferred on him.

In the course of a very active life Patterson published over a hundred scientific memoirs and in addition made several notable contributions to the history of chemistry. In some eighty papers dealing with various aspects of optical activity he accumulated a wealth of accurate measurement, often ingeniously contrived and exposing the multiplicity of factors which affect optical rotatory power. Assisted by various collaborators his critical studies in optical superposition (Part I, *J.*, 1905, **87**, 33; Part IX, *J.*, 1940, 862), his successful use of an optically active solvent for the study of intramolecular change (*Ber.*, 1907, **40**, 2564; Part I, *J.*, 1912, **101**, 26; Part VIII, *J.*, 1941, 606), and his extensive inquiry into the influence of solvents on the rotation of optically active compounds (Part I, *J.*, 1901, **79**, 167; Part XXXVII, *J.*, 1940, 290), all contributed their content of fact and experimental method to the enrichment not merely of his own field, but of others contiguous to it. On the other hand, his "Attempt to Harmonise the Relation between Temperature and Rotation of Optically Active Substances" (*J.*, 1913, **103**, 145) and the use he made of the Armstrong–Walker diagram (cf. *J.*, 1916, **109**, 1176; 1929, 2042) show the type of comprehensive system for which primarily he sought and by which, on occasion, he was able to correlate large sections of his measurements. It was foreign to his nature and standards to speculate on causes before the pattern of effects had been clearly defined and the persistence of his search reflects the tenacity of purpose which was at all times characteristic. To his eager mind and acute powers of observation projects of all sorts came crowding. Some of these he employed to help his associates towards the status of independent research, which he constantly advocated; others he developed, indulging especially his flair for mechanical contrivance or the design of apparatus (cf. *J.S.C.I.*, 1902, **21**, 456; 1924, **43**, 281; *J.*, 1927, 1717), but seldom allowing himself more than a brief departure from his main lines of inquiry. Latterly, however, an increasing portion of his time became absorbed in historical research.

Patterson was firmly convinced that the historical method, despite its difficulties, is the best approach to teaching science. This, with his passion for accuracy, was enough to ensure at least an occasional excursion into historical work, as instanced in his article on "Polarimetry" (Thorpe's Dictionary of Applied Chemistry, 1913) and by his essay on "Soda, Nicolas Leblanc and the French Revolution" (first read during his stay at Leeds and later published in *Proc. Roy. Phil. Soc., Glasgow*, 1924—1925, LIII, 113). Undoubtedly also his association with Ferguson and the respect in which he held that renowned bibliographer played their part, but the circumstance which ultimately set him deep in historical research was his introducing in 1924, for the benefit of his Honours Class, a course of lectures on the History and Philosophy of Chemistry. The honest teacher quickly became his own severest critic and sought the remedy in knowledge gained at first-hand. About this time books of Ferguson's unique collection,

purchased by the University, were made available and for long periods Patterson was lost to his colleagues behind a wall of dusty tomes, whence he would emerge triumphant, shedding drudgery and fatigue in the joy of a doubt resolved or a point of detail settled. His thoroughness won him general acclaim when the results of his work began to be published. His critical appraisal of Mayow appeared in *Isis* (1931, XV, 47) and was followed by a series of articles on Priestley, Richard Boyle, Van Helmont, Beguin, Vallensis and others, published mainly in *Annals of Science* (1936—1948). Much remains unpublished. In a special issue of *The Alchemist* (Glasgow University Alchemists' Club, **17**, June, 1942), produced in his honour at the time of his retirement, students, colleagues and authorities in his branches of research record their appreciation of his services to science and of his abilities as an administrator. There, too, a fairly complete list of his publications and many sidelights on his personality will be found and need not be duplicated here. Suffice it to say that the graceful and merited tributes from high quarters in two Continents show the esteem in which he was held during his lifetime.

He was a man of great charm, friendly, sincere and full of human understanding. He gave of his best to the young people in his care and was rewarded by a full measure of their respect and affection. In the lecture-room, as in conversation, his genuine scholarship, wide culture, and his precise, thoughtful exposition were sources of delight to his hearers, whilst in controversy, being committed and nothing loth, he maintained with equanimity a seemingly impregnable front. His outlook was tinged with conservatism (how strenuously, yet without rancour, he would have contested it!) but at times the schoolboy peeped from behind the professor's dignity and may be discerned even in his writings. Once, anticipating editorial disapproval of a less erudite form of reply to an opponent's argument, he blandly made his point with "—the *capita, ego vinco*; *caudae, tu perdis* principle is inadmissible in science." His fine sense of humour lightened laborious days, and the zest he had in life afforded the ready means of timely relaxation. Photography, cricket, golf supplied hobby and recreation, whilst most of his summer holiday was spent on his yacht, *Trilby*, which carried auxiliary motor-power in a temperamental mechanism named " Svengali." He was a member of the Clyde Corinthian Yachting Club.

Apart from departmental duties, made arduous by the effects of two World Wars and by a major share in the planning of new buildings to which he moved his department in 1940, Patterson served the University in many ways: he was Dean of the Faculty of Science (1923—1927), an Assessor on the University Court (1933—1941), and a member of numerous committees. He took a prominent part in the affairs of scientific societies in Glasgow. Becoming a Fellow of the Chemical Society in 1904, he served for a period as Member of Council (1921—1923) and later shared in the work of a Local Committee of which he was part-founder and the first Chairman (1937—1940).

In 1905 he married Miss L. P. Johnstone, daughter of the Rev. M. P. Johnstone of Greenock; she and a son survive him.

J. D. Loudon.

MARC TIFFENEAU.

1873—1945.

On Whitsunday morning, May 20th, 1945, Professor Marc Tiffeneau was struck down by apoplexy on the platform of the Gare du Nord, Paris, on his way to visit an invalid friend.

One of a family of eight, he was born on November 5th, 1873. His father and his grandfather owned a millinery business in Mony Oise, a few miles north of Paris. He attended the local Communal School and later the Marist Brothers College at Pont St. Maxence, Oise, where he passed the Bachelier examination at the age of sixteen. He then became an apprentice in the Pharmacie Frigaux and later in the Pharmacie Vigier, Paris. After three years' study in the Faculté de Pharmacie—gaining the gold medal in 1900—he filled posts in various hospitals as pharmacist and for a time was interested in the perfume business " de Laire." During this period he did much study and research and graduated as D.Sc. in 1907 and as M.D. in 1910. From 1924 to 1926 he was Professor in the Faculty of Science and from 1926 to 1944 in the Faculty of Medicine, and during 1939—1940 was Dean in the latter Faculty in the University, Paris.

Tiffeneau was a man of boundless energy and gave much help to many societies—Société chimique de France (1897, 1913 Council, 1920 Joint Secretary, 1938 Vice President); Société

de Pharmacie de Paris (1909, 1918 Secretary, 1935 President); Société de Chimie biologique (1914, 1922 President); Société de Thérapeutique (1911, 1931 President).

Among the honours conferred upon him were Lauréat de l'Académie des Sciences; 1911 Prix Jecker (divided), 1923 (whole); Chevalier 1922, Officier 1938, de la Légion d'Honneur.

In addition to French societies, he belonged to many others including the Chemical Society, London, of which he became a Fellow in 1920.

In his youth Tiffeneau had become a great friend of Ernest Fourneau (later Professor) and they gathered a group of enthusiastic young chemists, the group being entitled " Molécule." This group enabled the Société chimique de France to survive the 1914—1918 War.

The friendship of Fourneau with Tiffeneau lead to the marriage of the latter to Fourneau's sister. In his biography of Tiffeneau (*Bull. Soc. chim.*, 1948, [v], **15**, 905), Fourneau speaks of the delightful home life and particularly of the " mélomanes enragés " (mad musicians). Gustave Roussy (*Presse Med.*, 1945, **53**, 438) tells the story of Tiffeneau, a keen admirer of Wagner, pawning the gold medal (Pharmacie) he had just gained, in order to pay his travelling expenses to Bayreuth !

As previously mentioned, Tiffeneau devoted his time between 1889 and 1900 mainly to pharmaceutical work, but during the rest of his life there came from him either alone or with collaborators a steady stream of investigations in pure chemistry and to a smaller extent in physiological chemistry. The records of these—some 250 in number—appear mainly in the *Comptes rendus* and in the *Bulletin de la Société chimique de France* (112 papers) and others in the appropriate pharmaceutical, biological, and medical journals, a full list being given in Fourneau's biography (see above).

His first investigation, on anethole, was carried out in collaboration with Professor M. Béhal (*Compt. rend.*, 1901, **132**, 561) and gave proof that Bougault's idea that anethole has a trimethylenic chain is wrong and showed that its oxidation product has an allyl chain : $CH_3O{\cdot}C_6H_4{\cdot}CH{:}CH{\cdot}CH_3$. This was the beginning of the long series of researches on molecular transposition, that is change within the molecule, the migration of carbon radicals causing profound modifications. Typical examples are the following, in which hydriodides split off hydriodic acid :

$$C_6H_5{\cdot}\underset{\displaystyle CH_3}{C(OH)}{\cdot}CH_2I \longrightarrow CH_3{\cdot}CO{\cdot}CH_2{\cdot}C_6H_5 + HI$$

and

$$C_6H_5{\cdot}CH(OH){\cdot}\underset{\displaystyle C_6H_5}{CHI}{\cdot}CH_3 \longrightarrow CHO{\cdot}\underset{\displaystyle C_6H_5}{CH}{\cdot}CH_3 + HI$$

giving evidence of the migration of phenyl and methyl groups; others might be given showing the elimination of water from hydroxyl groups.

He introduced the terms " affinity capacity " of a radical, meaning the maximum quantity of affinity which it possesses and can exchange with an adjacent atom, and " migratory aptitude," meaning the relative ease with which a radical detaches itself from the atom to which it is attached.

The substances upon which Tiffeneau worked included ethylenic compounds, halohydrides, epoxides, glycols, vinylic alcohols, etc.

Space does not permit more than a mention of his physiological chemical work, but C. Heymans (*Arch. int. Pharmacodyn.*, 1945, LXXI, 202) recalls " ses belles recherches " on the relations between the chemical constitutions of new products, which he synthesised, and their pharmacological actions; on fundamental work on hypnotics and anæsthetics; and on alkaloids such as caffeine, digitalin, hordenine, and others.

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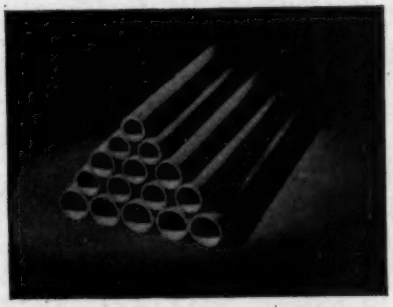

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